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Art. IV.—*Relationship of Insects to Parasitic Diseases in Stock.*

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(With Plates II.-VIII.).

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Part I.—The Life History of Habronema muscae, H. microstoma, and H. megastoma.

Introduction.

Although the life history of *Habronema muscae* has been known for some years as a result of the investigations made in U.S.A. by Dr. B. H. Ransom, there are no records of any such investigations in Australia, where variations tend to occur in the case of certain parasites owing chiefly to climatic and associated conditions.

Of the life histories of Habronema microstoma and H. megastoma nothing appears to be definitely known either in Australia or elsewhere.

In the adult stages these species of Habronema are well-known as more or less common stomach parasites of the horse, their occurrence under ordinary conditions being generally believed to be of little detriment to the health of the host. During recent years, however, more attention has been directed to these parasites in Australia as a result of the discovery here of larval Habronema, or an allied form, as the supposed causative agents of pathological conditions of the horse known as "Habronemic granulomata" and "Habronemic conjunctivitis." Further, splenic and stomach abscesses of the horse, due to H. megastoma, appear to have become of more frequent occurrence during the past few years, and there is reason to believe that under certain conditions the mortality caused in stock is considerable. These considerations then emphasised the desirability of testing under local conditions the life history of Habronema muscae, and of acquiring a knowledge of the life histories of other allied species.

Technique.

Among the first essentials for this work were means of obtaining (1) a pure culture of the parasite under investigation, and (2) absolutely "clean" flies, i.e., flies quite uncontaminated by any sort of helminth infection.

The former of these proved quite easy after some practice. All nematode eggs and embryos used in these experiments were obtained directly from gravid females, each of which was specifically determined whilst alive. After a thorough washing in saline solution these adults were placed in a watch-glass with a small quantity of sterilized normal saline, and cut into fragments between two needles, the contents of the glass were then washed off with the same fluid into a 4-oz. wide-mouthed bottle nearly full of fresh horse faeces, previously sterlized in the ordinary way in an autoclave for twenty minutes under a steam pressure of twelve pounds to the inch.

The second essential was finally secured by the adoption of methods which are here given somewhat fully in the hope that they may be of use to other workers, as a considerable amount of time had to be spent in perfecting the technique of breeding the flies and handling and preparing the worm larvae, as also in preliminary observations and experiments before the more systematic work of the investigations could be commenced.

Musca domestica.

Museum jars 8 in. high, and having an opening of 4 in. x 2 in., were obtained for the reception of the nidus. A sleeve to fit closely over these jars was then made by taking a piece of wire-gauze (12 meshes to the inch), about 8 in. wide by 14 in. long, and wrapping it tightly around the jar, while in this position the overlapping edges of the gauze were secured with solder and strengthened by a narrow strip of tin soldered along the whole length of the overlap, the top and bottom edges of the sleeve being similarly strengthened. In this way a sufficiently rigid and closely fitting, but removable, cage was made, which could be slipped over one edge of the jar, its outer open end being closed by a piece of paper folded over the edges and secured with a rubber band.

A strip of tin $1\frac{1}{2}$ in. wide and of sufficient length was then laid across the middle of the glass jar at the open end, bent sharply down each side for a distance of $1\frac{1}{4}$ in., then turned sharply upwards and snipped off about $\frac{3}{4}$ in. from the bend. This apparatus served as a clip to hold the gauze cage in position, and also as a receptacle for food.

In starting an experiment the jar is two-thirds filled with previously sterilized and cooled horse faeces, and the clip fixed in position, whilst the flies are caught in the vicinity of the stables in an ordinary butterfly net, with the end of the bag secured by a short piece of twine instead of the usual permanent stitching. As soon as a sufficient number of flies of the required species are caught, they are forced to the extreme end of the net by a few rapid sweeps, and imprisoned there by a few twists of the bag behind them, the twine is then untied, and the end of the net inserted well into the open end and towards one side of the otherwise closed gauze cage, which is then slipped over the mouth of the jar and forced down until its lower edge rests firmly in the metal clip. In a few minutes the flies find their way out of the open end of the net and congregate at the top of the cage. The cage is then tipped up sufficiently to permit of the withdrawal of the net and again forced down until it rests in the clip, the free ends of which are pressed firmly against the sides of the jar to hold the cage in position.

The best results were obtained when the flies were fed soon after being placed in captivity. For this purpose blood, sugar and water, cow's milk, and a preparation of dried separated milk, with the addition of water, were tried. The latter being readily obtainable at all times, and easily prepared, was found to be the most satisfactory. The liquid food was conveyed to the strip of tin across the mouth of the jar by means of a pipette passed through the meshes, the cage was then placed in the sunlight, or, in dull weather, near an electric radiator.

As a rule, sufficient eggs were deposited during the first day of confinement; if not, the flies were again fed and left for another day. When sufficient eggs were secured, the flies were liberated or removed in the gauze cage to another similar breeding jar, and the first jar covered with paper retained in position by means of a rubber band.

No special precautions were taken to maintain the eggs and larvae at a uniform temperature except that, as a rule, during the winter months they were kept in an improvised incubator, or near the electric radiator, at a temperature ranging from 20°C. to 28°C. During the summer months artificial heating was not resorted to.

On alternate days the larvae were supplied with freshly sterilized faeces, sometimes added to the top of the earlier supply, but more often placed in a clean jar. In the latter case the larvae were separated from the old faeces by merely turning the first jar into

another jar containing a few inches of fresh matter. In a few minutes the larvae migrated to the newer material, leaving the old to be removed and replaced by a more abundant supply of fresh food.

In some cases the pupae were removed from the faecal matter, washed in sterile physiological salt solution and transferred to moistened sterilized sand, but as this method did not appear to possess any advantages over the simpler procedure of allowing them to remain in the jar in which they had developed, it was discontinued.

On the appearance of the first flies the metal clip and gauze cage were fixed in position over the jar, and within a few hours sufficient flies were secured to breed from. The cage containing the newly emerged flies was then rapidly fitted to a clean empty jar, and food was introduced into it in the manner described.

Food was given once or twice daily for about eight days, during which period the flies were transferred on alternate days to clean cages and jars, and were kept as far as possible in a warm, sunny situation by day and near a radiator at night. Under these conditions they mated in four to six days after their emergence from the pupae and oviposited about four days later. On the eighth day the cage and its contents were transferred to a clean jar, containing freshly sterilized faeces, to which the flies had access until a sufficient number of eggs were deposited. The flies were then transferred to another similarly prepared jar for the production of more eggs, whilst the jar containing the first batches of eggs was covered with paper and transferred to a warm situation. The eggs hatched 12-24 hours later, producing larvae which, like their parents, had been reared exclusively on sterilized matter. It is obvious that any chance of helminth infection in these "clean" larvae is almost, if not quite absent, as shown also by the negative results of the examination of flies bred on such sterile faeces, and by the invariable absence even of moulds and such like from the jars. Such larvae were used in all the experiments in which this species (Musca domestica) were concerned, excepting where the contrary is stated. It may be mentioned here that the writer has succeeded in rearing four generations of flies in the laboratory under these methods.

Stomoxys calcitrans.

The methods employed for obtaining the larvae of Stomoxys calcitrans for experimental purposes differed from those outlined above only in the following details:—The adult caught flies did not

require to be fed during their captivity, and larvae of the first generation only were reared, and used in all the experiments with this species, owing to pressure of time when these flies and the Helminth material were obtainable.

A.—Habronema muscae, Carter.

1.—Historical.

A full account of the life-history of Habronema muscae, Carter, known since 1861 as a parasite of the House-fly (Musca domestica), was published by Dr. B. H. Ransom in 1913. As a result of his investigations it was shown that the life-history of this nematode is one requiring for its completion a simple alternation between two hosts, a vertebrate harbouring the adult parasite and an invertebrate harbouring the larval stage.

From horse faeces collected in the streets of Colorado Springs, U.S.A., on August 9th, 1911, Ransom bred flies (M. domestica) from which in the larval, pupal, and adult stages, he obtained a series of nematode larvae in successive stages of development commencing with a stage measuring about 0.4 mm. in length, from a fly larva, and reaching a maximum length of 3.2 mm. in an adult fly. On September 11th, 1911, he found amongst numerous filarioid worms collected from the stomach of a horse, larval worms in the same stage of development as those from flies. The adult worms from the horse's stomach were all of one species, and the younger stages formed a series between the adults on one hand and the larval Habronema muscae from flies on the other, thus establishing the fact that Habronema muscae of the fly, Musca domestica, is the larval stage of a parasite, the adult stage of which occurs in the stomach of the horse.

This parasite was shown to be similar to, but distinctly different from, a species found in the stomach of the horse, and known since 1886 as Spiroptera microstoma (Schneider), now correctly designated Habronema microstoma (Schneider).

In a further account of the development of the larvae of Habronema muscae within the body of the fly, Ransom gives details and drawings of six definite stages through which the parasites pass before reaching the stomach of the definitive host, to which stages reference will be made later on.

Seurat (1916, p. 321) considers that Ransom is quite wrong in describing these different steps in the development of the larval Habronema muscae within the fly (pupa and adults), as true stages,

this term being more correctly used to designate one of the five stages of increasing complexity of development characteristic of nematodes, each of these being separated from its predecessor by a distinct moult.

The same author (1912, p. 78) records the occurrence of both Habronema muscae and Habronema microstoma in horses and mules in Algiers.

Work upon somewhat similar lines to my own is being carried out by Mr. L. B. Bull, B.V.Sc., at the Government Pathological Laboratory, in Adelaide. A preliminary manuscript account of this Mr. Bull has very courteously allowed me to read while in the press, and also he has discussed certain points with me.

2.—The Adult.

Until very recently the presence of Habronema muscae in the stomach of horses in Victoria has not been recognised, although H. megastoma and H. microstoma have been well known. There is little doubt, however, that this has been due to a lack of distinction between H. muscae and H. microstoma, for since this investigation has been in progress, H. muscae has been found to be much more common than H. microstoma, and some specimens hitherto regarded as H. microstoma have turned out to be H. muscae on closer examination.

In the adult stage the parasite (H. muscae) occurs very frequently in the stomachs of horses in Victoria. A census of the parasites found in 39 horses' stomachs which were examined during the progress of this work shows that 33 of these harboured H. muscae. Further reference will be made to this in the general discussion later on.

In view of the excellent full description given by Ransom of the structure of this parasite, it is quite unnecessary here to attempt to add anything to it.

So far as is known, this species acts similarly to H. microstoma in its host.

3.—Record of Experiments, and Special Observations.

A. To determine the relationship of H. muscae to Musca domestica as an intermediate host.

Experiment No. 1.

Embryos from numerous H. muscae were liberated in sterile faeces at noon on October 30th, and incubated at a temperature

of 22° C. until noon on November 7th (eight days), when Musca domestica larvae (two days old) were liberated in the vessel. On November 20th and 21st fifteen flies were examined, ten of which were infected with from two to fifteen larval Habronema each. About half the total number of parasites were enclosed in cysts in the abdomen, the remainder were free in the abdomen, thorax, or head.

Experiment No. 2.

This experiment was similar to No. 1, excepting that the embryos were incubated for a period of 42 hours only (from 4 p.m. on November 20th to 10 a.m. on November 23rd). The fly larvae were of the same age and character as those used in Experiment No. 1 (i.e., two days old). Between November 26th and December 8th, 16 larvae, 12 pupae and 15 flies were examined, of which 1 larva, 1 pupa and 4 flies contained Habronema larvae.

Experiment No. 3.

Embryos from 20 H. muscae were liberated in faeces with a number of Musca domestica larvae (three days old), at 3.30 p.m. on November 26th. Between December 4th and 10th, 29 pupae and 12 flies were examined, of which 9 pupae and 5 flies were positive for Habronemic larvae. The infected pupae contained four or five parasites each, and the infected flies two to twelve parasites each. Twelve of the total number of parasites found in the flies were enclosed in cysts.

Experiment No. 4.

Embryos from numerous adult H. muscae were liberated in faeces at 4 p.m. on November 28th, with fly larvae then four days old. Between December 5th and 7th 1 pupa and 38 flies were examined, of which the pupa and 3 flies were positive. The pupa contained five parasites.

B. To determine the possibility of Stomoxys calcitrans acting as an alternative intermediary host.

In both these experiments Musca domestica was used as a control as to the viability of the Helminth embryos.

Experiment No. 5.

Embryos from ten worms were incubated in faeces from December 21st to December 27th, when larvae of Musca domestica and Stomoxys calcitrans, which hatched about December 18th, were

liberated in the vessel containing them. The Stomoxys larvae produced only one fly (January 7th), the result of examination of which was negative. Between January 7th and 8th, 34 Musca domestica flies were examined, 21 of which were positive and 13 negative for Habronema. The abdomen only of each of these Musca domestica flies were examined, the number of parasites found in each varying from four to sixteen. Both species of fly larvae used in the experiment were the progeny of eggs laid in the laboratory on sterilised faeces by naturally bred flies.

Experiment No. 6.

After a period of incubation in faeces, from 11 a.m. on January 11th to 11 a.m. on January 16th, larvae of Musca domestica and Stomoxys calcitrans were liberated in the embryo-infected faeces. These fly larvae hatched on January 7th, and were, therefore, nine days old when exposed to infection. On January 30th and the two following days, 15 Musca domestica flies were examined, all of which were positive for Habronema, the number of parasites in each varying from three to thirteen. On January 31st, and the five days following, 49 Stomoxys flies were examined, all of which were negative.

C. To determine the frequency of larval Habronema in Musca domestica, and their abundance and location in the body of the intermediary host.

(i) Free or caught flies.—During the period May-November, 1917, a record was kept of the number of flies (M. domestica) caught in the stables, and examined for the presence of Habronema larvae. It shows that 182 adult flies were examined, 14 of which were infected with Habronema muscae (?), as follows:—Eight harboured one parasite each in the head, one harboured two parasites in the head, one three in the head, four one parasite each in the abdomen.

(ii.) Although a similar record of laboratory bred flies has not been kept, it has been noticed that in the great majority of cases the abdomen only was infected, the proportion found in the head being much smaller than in the abdomen. This was particularly noticeable in heavily-infected flies.

The occurrence of Habronema larvae in the distal portion of the proboscis (haustellum) of Musca domestica has not been observed by the writer, although their occurrence in the proximal portion

(rostrum) is not unusual (Fig. 17). The greater proportion were found in the posterior part of the head capsule.

Ransom (1913, p. 13), states that the largest number of larval worms he found in an individual fly was eight, though, as he remarks, Carter (1861) noted as many as twenty in one fly.

The largest number of larvae, almost certainly H. muscae, found by the writer in a naturally infected fly (i.e., a fly caught in the stable) was three, but flies reared in the laboratory from larvae fed upon artificially infected faeces have produced a much greater number of parasites. Under these conditions 25 to 30 larval H. muscae in a single fly is not an unusual occurrence, while from one fly 72 of these parasites were obtained.

D. The viability of embryonic Habronema muscae in faeces.

It has been shown (Experiment No. 1, p. 8), that embryonic Habronema muscae removed from the uteri of gravid worms and incubated in sterilized faeces at a temperature of 22°C., may retain their viability and are capable of infecting fly larvae for a period of eight days.

In view of the results of similar experiments with the embryos of H. microstoma (see later Experiment No. 10, p. 26), it seems possible that this period may be considerably lengthened. Under the most favourable natural conditions it is conceivable that embryos may retain their viability for equally long periods.

E. To determine the period of survival of larvae removed from flies.

On January 7th, ten larval H. muscae from the abdomen of a fly were placed in saline solution in an open dish. On the following day two of the larvae were motionless and apparently dead, three more died on each of the two following days, leaving two nearly motionless larvae, both of which succumbed before noon on January 12th. Thus none of the larvae survived more than five days, although under similar conditions Ransom (1913, p. 14) found the longest period of survival to be between five and eight days.

F. To determine the period of survival of larval H. muscae in dead flies.

Twelve flies which emerged on December 4th, and died on the 6th, were placed on moist filter paper in a covered Petri dish at noon on the latter date. Twenty-four hours later four were examined, two of which contained 13 living larvae, several of which were

however, motionless. At noon on the following day (December 8th) the remaining eight flies were examined, three of which harboured 12 larvae. Of these nine were dead and three succumbed a few hours later in saline solution. Thus the longest period of survival was about two days, thus confirming Ransom's observations (1913, p. 14).

G. To determine the possibility of escape of larval Habronema muscae from flies.

On many occasions living flies were placed in moistened Petri dishes with the object of determining whether, under such conditions, the parasites could escape either before or after the death of the flies. In no instance could larvae be found in the dishes, although from subsequent examinations it was proved that the flies were heavily infected.

Similar negative results were obtained in experiments in which infected flies were allowed to die and remain for periods up to two days in saline solution.

Development.

The eggs and developing embryos, as found in the gravid female, are shown in Fig. 1. The egg (Fig. 1a) measures from 0.04 mm. to 0.05 mm. in length by 0.01 mm. to 0.013 mm. in breadth. Progressive stages of development are shown in Fig. 1, b, c, d, e, and f, in all of which the embryo is enclosed in a thin elastic sheath or egg-shell. In the stages shown in Fig. 1, e and f, two or three nuclei are usually seen, the largest being about 0.033 mm. to 0.036 mm. from the anterior end, the others at distances varying from 0.015 mm. to 0.035 mm. from the posterior end. A clear space surrounding what appears to be the rudimentary pharynx is rarely, though very indistinctly, seen. What appears to be a horn-like process at the anterior end can be made out with difficulty in the stage shown in Fig. 1, f.

The dimensions of the specimens figured are as follows :—(a) 0.043 mm. long by 0.013 mm. wide, (b) 0.052 mm. long by 0.0132 mm. wide, (c) 0.053 mm. long by 0.018 mm. wide, (d) 0.069 mm. long by 0.009 mm. wide, (e) 0.075 mm. long by 0.007 mm. wide, with nucleus at 0.033 mm. from the anterior end. In d, e, and f, the measurements given are of the embryo only, exclusive of the enveloping sheath, which varies in shape and size with the movements of the embryo, and no allowance is made for the bent tail end.

As Ransom has stated (1913, p. 16) the embryos undoubtedly pass out of the body of the horse in the faeces. Fig. 2 shows an embryo

0.089 mm. long by 0.0066 mm. wide, which was found in fresh horse faeces collected in a stable yard on October 11th, 1917. While this and many other embryos found under the same conditions have not been identified, there is little doubt that they are some species of Habronema. In this embryo there was a conspicuous nucleus at about 0.036 mm. from the anterior end, and a smaller one nearer the posterior end. It was not enveloped in a sheath or shell.

Two other embryos, each enveloped in a sheath, found in the same lot of faeces, measured 0.0925 mm. and 0.09 mm. in length respectively. In each the sheath was rather closely applied to the body except at the posterior end, where it was held away by the curved tail. The outline of the head could not be clearly seen in either of the embryos, but it appeared to be as shown in Fig. 2.

Development of the embryo in faeces.

No apparent development of the embryo takes place during its passage through the intestines of the horse, the embryos found in the uterus being indistinguishable from those found in fresh horse faeces. A certain amount of development does, however, take place during the life of the embryo in faeces. Fig. 3 shows an embryo obtained from a gravid female, which, with many other embryos, was incubated in sterilized horse faeces for a period of six days at room temperature. This embryo, which measured 0.086 mm. long by 0.065 mm. wide, was enclosed in a sheath 0.109 mm. long. The pharnyx, followed by a bi-lobed clear area and a granular and nucleated mass along the middle line of the body, could be fairly distinctly seen. The large nucleus was 0.036 mm. from the anterior end. Other embryos examined measured from 0.072 mm. to 0.086 mm. long by 0.0066 mm. wide, each being enclosed in a sheath measuring from 0.0825 mm. to 0.109 mm. long.

It is not known whether the embryos enter the fly larvae forcibly or whether they are ingested passively. Ransom considers that the latter is the more plausible theory, and in this I concur.

The early stages of Habronema muscae in the intermediary host (Musca domestica).

In larvae of M. domestica.

The earliest stage in which H. muscae are known to occur in the larvae of Musca domestica is shown in Fig. 4. This embryo, which

is four and two-third days old, was found in the alimentary canal of a Musca domestica larva, then five days old. The embryo was one of many which had been taken from a gravid Habronema muscae, and incubated in sterilized faeces at a temperature of 22°C. for 42 hours, at the end of which period they and the faeces were offered to fly larvae, then 48 hours old.

The embryo measured 0.0905 mm. in length by 0.0066 mm. in breadth, and agreed closely with the specimen shown in Fig. 3, excepting that it was free and not enclosed in a sheath.

Great difficulty is experienced in finding these embryos in fly-larvae, owing to their small size and transparency, and the nature of the debris surrounding them. The occurrence of other species of nematode worms in fly larvae, which, as Ransom has pointed out, aggravates this difficulty, has been obviated by the technique employed in these experiments.

In pupae of M. domestica.

Nothing is known of the further development which takes place in the young nematode larvae until the stage shown in Fig. 5 is reached. The specimen shown in Fig. 5 measured 0.25 mm. in length by 0.05 mm. in breadth at the posterior end of the oesophagus. The shallow mouth cavity appears to be followed by the oesophagus, but this structure could not be clearly seen owing to the fact that the cuticle was separated from the body at the anterior end, showing that the larvae was in process of moulting. It will be noted that in general appearance, this larva (measuring 0.25 mm. in length) agrees closely with the stage designated Stage 1 by Ransom (1913, p. 16), which measures from 0.4 mm. to 0.45 mm. in length (see later figures).

The same fly pupa contained two other larvae, a younger one measuring 0.2 mm. in length, which was not in process of moulting, and an older one, measuring 0.65 mm. in length (see Fig. 9). The embryos which produced these larvae were obtained from a gravid H. muscae on November 26th, mixed with saline solution and a small quantity of sterilized faeces, and fed to fly larvae which hatched on November 23rd. The pupa was examined on December 3rd; the oldest parasite was therefore not more than seven days old.

The next stage in the development of the larvae is shown in Fig. 6. This larva, from a fly pupa, was found on December 4th in the same culture as those referred to in the preceding paragraph.

The shallow oral cavity is followed by a straight pharnyx, which

was surrounded by a somewhat clear space extending nearly to the body wall. Its total length was 0.3 mm. the oesophagus was 0.117 mm. from the anterior end, and the diameter at the anterior margin of the rectum was 0.054 mm.

On the same date another larva of similar appearance, measuring 0.27 mm. in length, 0.05 mm. in width near the rectum, and having an oesophagus 0.11 mm. long, was found in a pupa from the same culture.

The larva shown in Fig. 7 is in the same stage of development as that shown in Fig. 6. It measured 0.34 mm. in length, 0.04 mm. in width at about the posterior end of the oesophagus, 0.05 mm. in width near the rectum, and 0.04 mm. from the anus to the tip of the tail. There was a constriction in the intestine near the junction with the oesophagus, but in living specimens this was seen to be due to the movements of the intestine itself. That the process of moulting had commenced was shown by the presence of the old cuticular lining of the oral cavity becoming detached from the body.

Fig. 8 agrees with the stage figured and described by Ransom 1913, p. 17, under the designation Stage 1, i.e., the earliest stage of H. muscae definitely known to him to occur in Musca domestica. The parasite shown in Fig. 8 was found on December 6th in a fly pupa, resulting from a larva which hatched on November 24th, and had lived on a culture of H. muscae embryos in sterilized faeces since November 28th, the date on which the embryos were obtained from a gravid worm.

The larva (Fig. 8) measures 0.45 mm. in length by about 0.045 mm. in width at the anterior end of the intestine. The oesophagus increases in diameter from 0.01 mm. at the anterior end to about 0.02 mm. at the posterior end. A nerve ring could be seen indistinctly at about 0.07 mm. from the anterior end. The intestine was about 0.18 mm. in length, and slightly narrowed towards the posterior end.

It will be noted that the clear space surrounding the pharynx of both younger and older stages is not shown in this larva. The moulting condition of the anterior end, and the consequent effect upon microscopical appearance may possibly account for the apparent absence of this feature. These remarks may apply also to the larva shown in Fig. 5.

The parasite shown in Fig. 9 was found, as already stated, on December 3rd, in a fly pupa which harboured the larva represented by Fig. 5, and was not more than seven days old.

The most noticeable feature of its development compared with earlier stages was its greatly increased length in proportion to increase in width.

The length of this larva was 0.65 mm. and the width at the posterior end of the oesophagus 0.05 mm. The posterior end of the oesophagus was 0.23 mm. from the anterior end of the body, and about the junction of the first and second third of the oesophagus there was a fairly conspicuous group of rather large nuclei indicating the position of the nerve ring.

Excepting in a general increase in size no marked structural development was observed in the parasites shown in Figs. 10 and 11, which were found on December 7th in fly pupa from the same culture as that which produced the parasites shown in Figs. 5, 6, 7 and 9. These larvae were, therefore, not more than eleven days old.

The smaller parasite (Fig. 10) was 0.8 mm. in length, the larger (Fig. 11) about 0.83 mm. In each the posterior end of the oesophagus was about 0.28 mm. from the anterior end of the body, the maximum width of which was 0.05 mm. The distance of the nerve ring from the anterior end was about 0.1 mm. and that of the anus from the tip of the tail about 0.060 mm.

Further than a general increase in size, no marked development was seen in the larvae shown in Figs. 12, 13, and 13A, as compared with those of earlier stages.

Fig. 12 represents a larva found in a thin-walled spherical cyst in the abdomen of a fly pupa in which the adult fly was almost ready to emerge. The embryo from which it was derived was one of many obtained from ten gravid females on December 21st, and incubated in sterilized faeces at room temperature for a period of six days before being given an opportunity of infecting fly larvae. The fly larvae used in this experiment were about nine days old, and nearly mature when they were liberated on December 27th in the embryo-infected faeces. The resulting pupae were examined on January 3rd; the parasite was therefore about thirteen days old, and had spent not more than seven days of its life within the body of the fly larva.

A larva in the same state of development as that referred to in the preceding paragraph, is shown in larger scale in Figs. 13 and 13A. It was found on November 30th free in a fly pupa from the culture referred to in discussing the embryo shown in Fig. 4. The age of this parasite and the fly pupa was, therefore, nine days, the first 42 hours of which period had been passed by the nematode embryo in sterilized faeces apart from the fly larva.

The length of the parasite was about 1 mm. (1.07 mm.) by 0.05 mm. in width at the posterior end of the oesophagus, which was 0.35 mm. from the anterior end of the body. The intestine was 0.62 mm. in length, followed by a pyriform rectum. The operculum like apex of the rectum was about 0.06 mm. from the tip of the tail and nearly level with the cuticle of the body. The intestine was 0.015 mm. diameter at the base of the oesophagus, increasing to about 0.03 mm. at about 0.07 mm. from its anterior end, then decreasing to about 0.015 mm., which diameter was maintained to near its junction with the rectum.

These parasites were evidently in the stage figured and designated Stage 2 by Ransom (1913, p. 18). It must be remarked, however, that in both parasites referred to in the preceding text nuclei were plainly seen in the rectum (see Figs. 12 and 13A, whereas Ransom remarks of his larva of Stage 2, "nuclei like those seen in the wall of the remainder of the alimentary tract are absent from the rectum."

In adult M. domestica.

Very definite progress in development is seen in the larva represented by Fig. 14, which was found in an adult fly from the same culture as the parasite shown in Fig. 12 and discussed above.

The fly emerged and was examined on January 7th; the parasite was, therefore, seventeen days old. The first six days of its embryonic life were spent in sterilized faeces, with similar embryos only, and not more than eleven days in the body of its intermediate host.

It measured 1.435 mm. in length by about 0.043 mm. in width. The pharnyx, which was 0.0297 mm. in length, was surrounded near the anterior end by a somewhat clear space. The oesophagus was about 0.013 mm. in diameter at the anterior end, increasing very gradually to a minimum diameter of 0.0198 mm. at its base, which was 0.43 mm. from the oral opening. The conspicuous nerve ring, surrounded by numerous nuclei, was about 0.12 mm. from the anterior end of the parasite. A few small nuclei occurred in the body-wall and alimentary tract, but these were few and scattered.

No evidence of a spinous-tipped tail could be seen under the moulting cuticle of this or other larvae of about the same length and condition. This larvae would appear to be in a stage of development near to Stage 3 of Ransom.

Although larvae of this stage are usually found in adult flies, a similar parasite was found in a Musca domestica larva which had

been reared entirely on faeces from the rectum of a horse believed, after a post-mortem examination, to be infected with Habronema muscae only. The fly larva was ten days old at the time of examination, its growth and development having been retarded by low temperature and insufficient nourishment.

In the following stage the vacuole or clear space surrounding the pharynx disappears, but the anal operculum remains, thus agreeing with Ransom's description of his Stage 4 (1913, p. 20). The pharynx is much longer and distinctly wider than in the preceding stage. The oesophagus is also much longer. There is still no indication of the future spinous tip to the tail. A typical larva in this stage of development measured 1.848 mm. in length, by 0.05 mm. in width at the base of the oesophagus. The pharynx was nearly 0.043 mm. in length, and had an oral opening similar to that of the larvae represented in Fig. 15. The nerve ring and base of the oesophagus were 0.12 mm. and 0.64 mm. respectively from the anterior end of the body. The anus was about 0.079 mm. from the tip of the tail, the process of moulting was nearly complete. The worm was found in the abdomen of a fly from the same culture as the parasites shown in Figs. 12 and 14. The fly emerged, and was examined on January 9th; the parasite was, therefore, about nineteen days old, and had lived in its intermediate host for not more than thirteen days.

In the next stage (Ransom's Stage 5), the spines at the tip of the tail are seen under the cuticle and the rectum is distended as in earlier stages, and as recorded by Ransom (1913, p. 21). A larva in this condition was found on the same date in the same culture as that described in the preceding paragraph (i.e., a larva agreeing with Ransom's Stage 4). It measured 2.013 mm. in length by about 0.056 mm. in width at the base of the oesophagus. The pharnyx was nearly 0.043 mm. in length. From the anterior end of the body the nerve ring and base of the oesophagus were distant 0.122 mm. and 0.775 mm. respectively. This worm was found in a cyst in the abdomen, and was about to moult.

Fig. 15 represents a larva in the condition designated Stage 6 by Ransom, which is the final larval stage of Habronema muscae found in the fly. It was reared from a culture of embryos taken from a gravid female on January 11th, and incubated in sterilized faeces for five days (i.e., until January 16th), when the embryo-infected faeces were fed to fly larvae, which hatched on January 7th and emerged as flies on January 30th. The parasite was therefore nineteen days old, not more than fourteen days of which period were spent in its intermediate host.

It measured 2.376 mm. in length by about 0.05 mm. in width at the base of the oesophagus. The cylindrical pharnyx was 0.0495 mm. in length. The nerve ring and the base of the oesophagus were about 0.132 mm. and 0.92 mm. respectively from the anterior end of the body. The anterior end of the oesophagus measured about 0.0115 mm. across, and increased in width somewhat sharply about 0.208 mm. from the anterior end of the body to a maximum width, at the base of the oesophagus, of 0.039 mm. The tail tapers evenly and gradually to the rounded tip, which is covered with small spines (Fig. 16). Similar larvae are commonly found in the head, thorax, and abdomen of flies, and not infrequently within cysts in the last position.

The length of larval H. muscae in the final stage found by the writer in laboratory-bred flies ranges from 2.145 mm. to 2.541 mm., while the range for what are believed to be Habronema muscae (three specimens) found in naturally infected caught flies is 2.178 mm. to 2.244 mm. The range given by Ransom (1913, 21) for the latter is 2.6 mm. to 3.2 mm., the minimum being only 0.04 mm. less than the maximum length known to the writer to be attained by Habronema in the fly. This specimen from a culture artificially infected with embryonic H. muscae (?) was found (November 10th) in a fly five days after its emergence from the pupa.

All stages of the parasite excepting the earliest stage found in the fly larva (see Fig. 4) are known to the writer to sometimes occur in cysts.

Encystment is a condition believed to be assumed by the larval parasite when about to enter a resting period, and not a necessary condition in the process of development.

It has not been considered necessary at the present stage of this investigation to discuss in detail the minute internal developmental changes which take place in the embryonic and larval stages other than as indicated by the variations in the relative lengths and diameters of the parts shown in Table No. 2. The general structure of each stage is shown by the figures and the comments thereon, in this section the term "stage" being employed in a wide sense to indicate obvious steps in the process of development.

Summary and Discussion.

As will be seen, this investigation into the life-history of Habronema muscae is practically a confirmation and extension of the observations by Ransom, to which frequent reference has been

made in the preceding pages, and although the methods employed are entirely different, there are no essential details in the results upon which there is disagreement.

Thus, the embryos passed out in the faeces from the horse are taken up by the larvae of adults of Musca domestica, which have oviposited on the faeces, which remain infective in this respect up to at least eight days after leaving the rectum, the fly larvae being known to react to infection when forty-eight hours up to nine days old, and may be earlier and later. After a slight amount of development in the faeces (Figs. 2 and 3), the embryo of Habronema muscae enters the larva of Musca domestica (see Fig. 4). Then it continues to develop in the fly pupa through the various stages shown in Figs. 5-13A, and in the adult fly as seen in Figs. 14-16, in which condition it is ready to develop in the stomach of the horse, where such stages have been met with.

Ransom (1913, p. 15) has suggested the possibility of infection of the horse in three ways. Firstly, by ingestion of dead infected flies, which he considers perhaps a common source of infection. Secondly, by ingestion of the parasite in water or moist material. Thirdly by ingestion of the parasite after its escape from the fly whilst feeding upon the mucous membrane of the horses' mouth.

His experiments prove that possibly the second theory may account for an occasional infection, but that such infection is not a normal occurrence. My own experiments do not support this second theory at all, since as shown above, I have been unable to obtain any evidence of the escape of the parasite from the fly, though Mr. Bull informs me that he has found such larval parasites to have escaped from flies kept in tubes, whether from living or dead flies was not known.

Concerning the third theory, Ransom says that there is no evidence, as yet, that larval Habronema escape from the fly whilst the latter is feeding upon moist surfaces or matter. The evidence also is still not forthcoming as the result of my experiments.

From these and other observations made during the progress of these investigations, the present writer has no hesitation in expressing the opinion that the ingestion of both living and dead infected flies provides the normal means by which the larval Habronema finds its way into the horse's stomach.

That living flies and those which have recently succumbed are quite commonly ingested by horses from the drinking trough and manger is beyond question. On a frosty morning it is here a common occurrence to find numerous benumbed flies (both Musca

domestica and Stomoxys calcitrans) dislodged from adjacent walls in food and water containers provided for horses.

In the summer months the fodder will be found to be frequented by great numbers of flies of both species, Musca domestica predominating in both cases. That many of these are ingested can scarcely be doubted.

Johnston (1912, p. 76) records the occurrence of larvae of Habronema muscae in Stomoxys calcitrans and Musca domestica in Sydney, and in Musca domestica in Brisbane. If the parasite found by him in Stomoxys calcitrans was of the same species as those found in Musca domestica, the occurrence of the former in Stomoxys calcitrans would appear to be merely a rare accident, inasmuch as on two occasions a massive infection of faeces was supplied by me to both Musca domestica and Stomoxys calcitrans in the same jar, resulting in a heavy infection of the Musca domestica and an entire absence of infection in the fifty Stomoxys calcitrans examined.

B.—Habronema microstoma (Schneider).

1.—Historical.

In the adult stage Habronema microstoma has been known since 1866 as a parasite occurring in the stomach of the horse, but nothing has been known definitely of its life-history.

Von Linstow (1875, pages 195-197) found in the heads of Stomoxys calcitrans a nematode embryo and several larvae which he, assuming them to be of the same species, described and figured under the designation of Filaria stomoxeos. Noè (1913, p. 392) states that Filaria stomoxeos of Linstow is identical with nematodes found by him in Stomoxys, and considers Linstow's species to be an intermediate stage of Filaria labiato-papillosa of cattle (see Ransom, 1913, p. 9). Ransom further remarks that Noè's statements have been commonly accepted, but that it is impossible to judge definitely from the data given whether or not he (Noè) is correct in his opinion as to the identity of the nematodes, and as to their being intermediate stages of Filaria labiato-papillosa.

From Linstow's description Ransom is inclined to consider Filaria stomoxeos a species of Habronema rather than a larval form of Filaria labiato-papillosa, and he suggests the possibility of it being a larval stage of Habronema microstoma.

Other than these suggestions nothing is available as to the life-history of this form.

The Adult.

In the adult stages H. microstoma bears a very close similarity to H. muscae, but the species are distinctly different, and, after a little experience may be easily separated, even when living.

Although well known in Victoria, it is not so frequently met with as its near ally (H. muscae) as will be seen from the results of the census of the parasites found in thirty-nine horses' stomachs examined during the progress of these investigations (c.f. Table No. 9). Whereas H. muscae were found in thirty-three stomachs, H. microstoma were found in only fourteen. In all cases in which both species were found in the same stomach the former greatly outnumbered the latter.

It will be noticed in Table No. 9 that H. microstoma were not found in eleven stomachs examined in the months May—August.

As Ransom has fully described and figured the adult H. microstoma (1913, p. 27), it is unnecessary here to enter into details of the structural and diagnostic characters of the species.

Record of Experiments and Special Observations.

A. To determine the intermediary host or hosts of H. microstoma and the early life-history of the parasite.

The technique followed in these experiments has been fully described in the Introduction to this Report.

Experiment No. 7.

On October 25th embryos from twenty gravid H. microstoma were liberated in sterilized faeces with a number of "clean" four-days' old Musca domestica larvae. Twenty-two of the fly larvae were examined on October 29th and the first two days of November. One of them was found to harbour a single embryo similar to that seen in Figure 20. The remainder of the larvae were not found to be infected. Twenty-seven pupae and twenty-two flies from the same culture jar examined between October 31st and November 7th were not infected.

Experiment No. 8.

Embryos from five worms were liberated in faeces on November 26th, with a number of Musca domestica larvae then three days old. Five larvae, 57 pupae and 35 adult flies were examined between November 29th and December 11th, of which only one larva was found to be infected (November 29th). The embryo found in

this larva agreed with the one found in similar circumstances in Experiment No. 7, and with that found under similar conditions in Experiment No. 12 (see Fig. 20).

Experiment No. 9.

This experiment, commenced on November 28th, was similar to No. 1, excepting that the embryos were obtained from three worms only. Between December 4th and December 12th, 1 larva, 60 pupae and 25 adult flies were examined, none of which was found to be infected.

Experiment No. 10.

On December 3rd Experiments Nos. 7 and 9 were repeated, with embryos from seven worms fed to four-days-old fly larvae. Subsequently 15 pupae and 55 adult flies of Musca domestica were examined (December 17th and 20th), with negative results.

On December 18th, finding that the faecal matter was still heavily infected with living embryos of H. microstoma, then enclosed in sheaths, a number of two days old larval Stomoxys calcitrans were liberated in it. Forty-four adult Stomoxys flies, resulting from these larvae, were examined between January 7th and 10th, with the result that 21 were found to harbour from one to three larval Habronema each, while the remaining 23 were negative.

As will be seen later (Experiment No. 13), the difference in age of the larvae to which the embryos were fed cannot be supposed to have had any influence on the result here recorded for Experiment 10.

Experiment No. 11.

Embryos from about 30 gravid worms were liberated in faeces containing numerous five and six days old Musca domestica larvae on December 14th. Sixty-eight of the resulting fly pupae and adults were examined between December 17th and 24th, none of which was found to be infected.

Experiment No. 12.

Embryos from six gravid worms were liberated in faeces on December 21st, and incubated at room temperature until December 24th, when a number of Stomoxys larvae, then six days old, were added to the culture. On December 27th a fly larva was examined and found to harbour one embryo (see Fig. 20) comparable in structure with those found in Experiments 7 and 8, with M. domestica larva. Five pupae were examined on January 3rd and 4th,

and sixteen adult flies from January 10th to 16th, all of which were infected, the number of larval nematodes in each varying between 4 and 50, averaging about 25.

Experiment No. 13.

After a period of forty-eight hours' incubation at room temperature, a culture of previously sterilized faeces containing embryos from twenty specimens of Habronema microstoma was infected on January 16th, with nine-day-old larvae of Musca domestica, and with Stomoxys calcitrans larvae of the same age. The majority of the Musca larvae and some of the Stomoxys had already ceased feeding, consequently infection of the former was not expected. Only two Musca domestica flies emerged (January 27th and 28th), neither of which harboured nematode larvae. On the other hand, two Stomoxys larvae which were examined on January 2th, harboured four and five parasites respectively, while each of three pupa examined on January 23rd and 29th contained upwards of thirty-five parasites. Other Stomoxys pupae were examined between these dates, each of which was heavily infected.

Experiment No. 14.

Embryos from six worms were liberated in sterilized faeces on January 22nd, and incubated at room temperature until January 29th, when Musca domestica larvae, then five days old, were added to the culture. Twenty-three flies emerged on February 11th and 12th, of which number one only was infected. This parasite (Fig. 26) was evidently malformed.

B. To determine the frequency of larval Habronema in Stomoxys calcitrans, and their abundance and location in the body of the intermediate host.

(i.) Free or Caught Flies.—During the period May to November 63 flies, 10 pupae and 12 larvae of Stomoxys calcitrans were collected in the Institute grounds and examined for the presence of Habronema. Of this number only one fly was found to be infected (May 4th). The parasite, which was located in the abdomen, measured about 1.5 mm. in length and agreed with typical examples of larvae of H. microstoma, as found in flies bred and infected in the laboratory.

(ii.) Laboratory bred flies showed a very high percentage of infection, as will be seen by reference to Experiments No. 10, page

31; No. 11, page 31; No. 12, page 31; No. 13, page 32; No. 14, page 32.

In the first of these experiments (No. 10), 47.7% of the flies examined were infected, and in the remaining four 100% of those examined harboured from a few to 60 parasites each. In most cases the majority of the parasites were located in the proboscis and head, particularly in heavily infected individuals. The following record of the location of parsites is fairly typical:—Fly (a) harboured 45 parasites, of which 10 were located in the proboscis, 20 in the head, and 15 in the thorax and abdomen; fly (b) harboured 36 parasites, of which 15 were found in the proboscis and 21 in the head; fly (c) harboured 27 parasites, of which 12 were in the proboscis, 14 in the head, and 1 in the thorax; fly (d) harboured 30 parasites, of which 15 were in the proboscis and head, and 15 in the thorax and abdomen; fly (e) harboured 60 parasites, of which 35 were found in the proboscis and head and 25 in the thorax and abdomen.

Encysted larvae are frequently found in the abdomen of the infected fly. Generally a cyst contains a single larva, and there may be several cysts in one fly. Larger cysts containing more than one parasite are seldom found. One Stomoxys fly, however, which was found to harbour over 60 parasites, contained three cysts in the abdomen, one of which enclosed one parasite, one five parasites, and one seven parasites.

C. To determine the viability of embryonic Habronema microstoma in faeces.

It has been shown (Experiment No. 10, p. 31, that embryonic H. microstoma may survive for a period of fifteen days in sterilized faeces and still remain capable of infecting fly larvae. In this experiment embryos were incubated in faeces from December 3rd to December 18th, before being exposed to ingestion by Stomoxys larvae. All embryos found on the latter date were enclosed in sheaths. The resulting flies were examined between January 7th and 10th, when over 47% were found to harbour from one to three parasites each.

Under favourable natural conditions the period of viability is possibly quite as long—indeed there is some reason to believe that a fairly long period of viability in faeces is necessary for the propagation of the species. This aspect of the life-history of H. microstoma is referred to more fully elsewhere in this report. (See page 44).

D. To determine the period of survival of larvae removed from flies.

During the afternoon of January 16th, 25 H. microstoma larvae from the heads of two Stomoxys flies were placed in normal saline contained in a small covered Petri-dish and examined daily until January 20th. Two were dead on January 17th, five on January 18th, twelve on January 19th, four on January 20th, leaving two living at noon on the last date. No examination was made on January 21st, but both were found dead early on January 22nd.

E. To determine the period of survival of larvae in dead flies.

A Stomoxys fly which emerged on January 14th was kept in a wire-gauze cage until January 16th, when it died. It was then placed on moistened paper in a covered Petri-dish, where it remained for forty-eight hours. On dissection the head and the proboscis were found to contain 35 dead larvae.

F. To determine the possibility of escape of larval H. microstoma from flies.

Stomoxys flies were frequently placed in moist, and in dry, tubes for periods up to thirty hours, to determine whether under such conditions the parasite would escape either before or after the death of insects. Although the flies were subsequently proved to be heavily infected, in no instance were parasites found free in the tubes.

Time has not permitted, so far, of much experimental work to determine whether or not larval H. microstoma may be carried into a wound made by a Stomoxys fly during feeding operation, but on January 12th attempts were made to induce Stomoxys flies (afterwards proved infected) to bite horses by placing them on various parts of the skin, including a bare patch on the back. In some cases there was a determined attempt on the part of the flies to bite, but strangely enough none succeeded in penetrating the skin. Two of these experimental flies were subsequently dissected and found to harbour 12 and 15 parasites respectively in the proboscis and 14 and 25 respectively in other parts of the head.

The escape of larvae from freshly severed heads in normal saline has been observed frequently. The exit of the parasite is made very rapidly, either from the tip of the proboscis, or by forcing its way out between the labium and the labrum-epipharynx. Photo-

micrographic Figs. 34 and 35 show larvae escaping in both ways.

It seems possible in view of the above facts and the well known piercing power of the proboscis in Stomoxys calcitrans that the failure of the specimens to pierce the skin in the above experiment was due to the proboscis being clogged by the larval parasites, twelve in one case and fifteen in the other.

G. To determine whether from a mixed infection of Habronema muscae and Habronema microstoma in sterilized faeces any selective action is present between the parasites and a particular species of intermediate host.

All the evidence adduced as a result of the experiments and observations recorded in the preceding pages of this report is in the direction of establishing the fact—(1) that Habronema muscae has Musca domestica for its only intermediary host, (2) that H. microstoma has Stomoxys calcitrans for its principal intermediary, (3) that Musca domestica may act, very rarely and in small measure —under experimental conditions, at any rate—as an alternate intermediary host for H. microstoma.

It appeared desirable therefore to carry out further experiments to determine the selective activity (if any) shown by Habronema embryos of a particular species for a particular species of intermediary, and especially the relationship of Stomoxys calcitrans to Habronema musca and the relationship of Musca domestica to Habronema microstoma when given a free choice. With this object in view the following experiments were carried out:—

Experiment No. 15.

On January 21st embryos from three gravid females of H. muscae and H. microstoma were liberated in sterilized faeces, and on the following and subsequent days larval Musca domestica and Stomoxys calcitrans, then three days old, were fed upon the infected matter. The first 28 Musca domestica flies emerged on February 4th, of which 15 were infected and 13 were free from parasites, the number of parasites in infected flies varying from one only in each of seven flies to four in each of three flies.

The first six Stomoxys flies emerged on February 11th, all of which were found to be heavily infected, i.e., not less than 60 parasites in each. Details and measurements of some of these larvae are given in Tables 5 and 6.

In Table 5 particulars are given of six larvae from Musca domestica flies (February 4th). The measurements of specimens

1 and 2, when compared with those of undoubted H. muscae in the same stage of development (Table No. 1), agree closely with that species, to which they undoubtedly belong.

On the other hand, the measurements of Specimens 3-6 (Table No. 5) do not agree with those of Specimens 1 and 2 of the same table, but they agree fairly closely with undoubted specimens of H. microstoma in a similar stage of development (see Table 2), and there is no doubt in the writer's mind that they are referable to the last-named species and to the stage of that species preceding the appearance of spines under the cuticle at the tip of the tail.

It was not noted whether both species of Habronema occurred in any individual fly. In Table No. 6 measurements and details are given of eleven larval Habronema from Stomoxys calcitrans flies bred from the same culture (February 11th). A comparison of these measurements with those of undoubted H. microstoma (Table No. 2), and H. muscae (Table 1) leaves little doubt but that they are referable to the former species only.

Experiment No. 16.

Embryos from gravid H. muscae and H. microstoma were liberated in sterilized faeces on January 11 and 14th respectively, and incubated until January 16th. On January 14th and subsequent days larval Musca domestica and Stomoxys calcitrans, which hatched on January 7th, were fed upon the infected matter and of the resulting flies one M. domestica and twenty S. calcitrans were examined and found to be infected on January 29th and 30th.

The measurements of four Habronema larvae from the Musca domestica fly are given in Table No. 7, the measurements of the remaining three being omitted on account of their agreement with Specimen No. 2 in the above table. It will be seen by comparing these measurements with those shown in Tables Nos. 1 and 2, that the larvae found in this fly were almost certainly H. muscae. If a further comparison is made between Table 8, which gives the measurements of 6 Habronema larvae from Stomoxys flies (30th January), and Tables Nos. 1, 2 and 7, it will be found that the evidence is strongly in favour of these larvae (Table 8) being referable to H. microstoma.

These experiments confirm the fact that the final larval stage of H. microstoma in the fly may be attained in the body of Musca domestica as well as in Stomoxys calcitrans, while previous experiments (7, 8, and 14) seemed to show that such was not the case.

Development.

The eggs and embryos of Habronema microstoma as found in the adult worm are illustrated in Fig. 18. The egg (Fig. 18a) measures from 0.04 mm. to 0.05 mm. in length by about 0.01 mm. in diameter, and is similar to that of H. muscae. Fig. 18, b and c, show progressive stages in which the developing embryo is clearly seen within the egg-shell. The final stage (Fig. 18 d), within the uterus of the female is reached when the embryo attains a length of from 0.085 to 0.105 mm., and a diameter of from .0055 mm. to 0.0075 mm. At 0.035 mm. to 0.045 mm. from the anterior end there is a more or less conspicuous nucleus and frequently two or three smaller nuclei may be made out nearer the posterior end. The form of the anterior end can be made out only with difficulty. There appears to be a rudimentary pharynx and a small horn-like process in front of the head, as in H. muscae, but the clear space sometimes seen surrounding what appears to be the pharynx in that species has not been observed in H. microstoma.

Development of the embryo in faeces.

There can be little doubt but that embryos leave the horse in the faeces, but it is not known whether they undergo any further development during their passage through the intestines. Definite development does take place, however, in the faeces after egestion, but whether this development is necessary before the embryo is capable of entering the fly larvae is not yet known, owing to the great difficulty of being sure of the species of the larvae found in the fresh faeces, material not having been available from a horse which could afterwards be known to have contained H. microstoma only.

Fig. 19 shows an embryo which was taken from the uterus of an adult worm on December 21st, and incubated in sterilized faeces until December 26th (see Experiment No. 12, p 31). The parasite, which was very active, was at the end of this five days' incubation 0.115 mm. long by 0.007 mm. wide. About 0.026 mm. from the anterior end there was a group of small nuclei which were not seen in other parts of the body, and at 0.053 mm. from the anterior end there was a much larger and more conspicuous nucleus. The sheath which enveloped it was very thin and elastic.

In another similar culture (Experiment No. 8, p. 30), in which the embryos had been incubated for a period of three days (Novem-

ber 26th to 29th), active embryos were found which measured about 0.1 mm. long by 0.0065 mm. wide. The group of small nuclei observed in the larger embryo referred to above were not made out, but a fairly large and conspicuous nucleus was seen in each about 0.045 mm. from the anterior end. The majority of these embryos were enveloped in sheaths, but others were free. On the same day and from the same culture a Musca domestica larva was found to be infected with an embryo measuring 0.099 mm. long by 0.0065 mm. wide, with a nucleus at 0.048 mm. from the anterior end, but otherwise agreeing with the forms found free in the faeces.

On December 27th an embryo (Fig. 20) was found in a Stomoxys larva which agreed very closely with the one shown in Fig. 19. Both embryos were from the same culture (i.e., Experiment No. 12, p. 31), but whereas the later (Fig. 19) was five days old, and had lived in faecal matter only, the former (Fig. 20) was six days old, and may have been harboured within the fly larva for not more than three days.,

The parasite shown in Fig. 20 measured about 0.115 mm. long by about 0.0066 mm. wide. A very large nucleus occurred at about 0.039 mm. from the anterior end, and numerous small ones could be seen occupying the middle line of the body for the greater part of its length. The tail was usually carried bent sharply over, but during the frequent snake-like movements of the body it was seen to be capable of the freest action. This embryo, like the other from the same culture, was enclosed in a sheath.

Succeeding stages in the development of the parasite are shown in Figs. 21, 22, and 23. These three, in addition to two embryos similar to the one represented in Fig. 20, were found on January 24th in a Stomoxys larva from the culture referred to in Experiment No. 13, p. 32. The latter two were located in the alimentary tract, and were not enclosed in sheaths, the others, Figs. 21, 22 and 23, were found in the fat-body separating the body wall from the alimentary tract.

The parasite shown in Fig. 21 was about 0.138 mm. long by about 0.02 mm. wide at the anus. The process at the anterior end noted in earlier stages was still evident. An oral opening could not be made out, but a clear space at the anterior end of the body indicated the presence of a pharynx. There was no visible alimentary tract, the whole of the body being apparently composed of a mass of nuclei of varying sizes, those in the anterior half being largest. The anal opening was closed by a rounded projection. Only a portion of the rectum could be seen clearly. The tail, which

was curved, tapered very abruptly, and ended in a finely pointed tip.

Fig. 22 shows a parasite evidently in a slightly more advanced state of development. It measured about 0.15 mm. long by 0.025 mm. wide at the anus, and was enclosed in a spherical cyst. The process at the anterior end, previously referred to, was still to be seen, although a change in the general outline of that end was observed. The clear space seen at the anterior end of the body in the preceding stage (Fig. 21) was not made out. The whole of the body was composed of nuclei, those about the middle being the largest. Excepting the rectum, which was large, there was no evidence of an alimentary tract. The rectum was much distended, but the anus, which was 0.026 mm. from the tip of the tail, was closed as in the preceding stage.

The larva seen in Fig. 23 showed still further development. In this the outline of the anterior end was still more irregular than in the preceding stage. A well-defined mass of large nuclei occupied the greater part of the body from near the anterior end posteriorly for a length of 0.082 mm. From the posterior end of this mass to the rectum these nuclei were somewhat smaller and more scattered and the rectum was rather smaller than in the preceding stage. The anus was 0.026 mm. from the tip of the tail, and was closed by a rounded projection as in earlier stages. The length of the parasite was about 0.175 mm.

Reference to Experiment No. 13, page 32, shows that these five parasites were ten days old when examined, and that the period of their existence in the Stomoxys larva could not have exceeded eight days. Presumably the two least developed forms found their way into the body of the intermediate host later than the three more advanced ones.

Larval stages comparable with those shown in Figs. 21, 22 and 23 are not known in H. muscae, but probably they do occur between the stages illustrated by Fig. 4 and Fig. 5.

The earliest stage in which a definite oral opening is known to occur is shown in Figs 24 and 25. This larva, together with several others in the same stage of development, was found on January 23rd in a newly-formed pupa from the same culture as the larvae shown in Figs, 21, 22, and 23 (i.e., Experiment No. 13). It was therefore nine days old, not more than seven days of which period had been spent in the body of the fly larva. It measured 0.24 mm. long. The oral cavity was apparently closed at its junction with the pharynx (?). The body, which was 0.24 mm. long, was narrowest

at the anterior end, and increased gradually to the anus, at which point it was 0.05 mm. in diameter. The tip of tail which was bluntly pointed was about 0.05 mm. from the operculum-like projection of the anus. This larva was apparently somewhat less developed than the larval H. muscae shown in Fig. 5, which measured 0.25 mm. long, and which was probably some days younger (c.f. page 22).

Fig. 26 shows a larva in a somewhat similar stage of development, which was found on February 12th in the abdomen of a Musca domestica fly (see Experiment No. 14, page 32). It measured 0.25 mm. long by 0.049 mm. wide. The oesophagus, which was considerably twisted, joined the intestine about 0.082 mm. from the rectum. The anterior end was partly enveloped in a portion of the cast-off cuticle, a portion of which adhered also to the posterior end. The rounded tail, the general appearance of the parasite, and the occurrence of such an undeveloped stage in a mature fly of a species not apparently normal for this worm suggests that this specimen was an abnormality.

Figs. 27 and 28 show the next step in the development of the larvae. The lower part of the pharynx is now surrounded by a clear space or vacuole, a feature which has not been observed in larval H. musca of less than about 0.65 mm. in length, whereas the worm figured in Figs. 27 and 28 measured only 0.247 mm. long by 0.04 mm. wide at the rectum. The form of the oesophagus and intestine are now well defined, and the large nuclei have almost disappeared from the alimentary tract. Small nuclei only are dispersed through the oesophagus, intestine, and body wall, but those in the rectum and posterior end are considerably larger. The body is relatively longer and narrower than in earlier stages.

At the base of the oesophagus, which was 0.1 mm. from the anterior end, the diameter of the body was 0.036 mm. The anus, still closed as in the earlier stages, was 0.033 mm. from the tip of the tail. That the worm figured was undergoing a moult was shown by the presence of the partly discarded cuticular lining of the oral cavity.

This parasite was found on January 4th in a Stomoxys pupa which had developed in the same culture as the embryo illustrated by Fig. 20 (i.e., Experiment No. 12, page 31). It was therefore fourteen days old, and had been harboured by the intermediate host for a period not exceeding eleven days. This pupa contained upwards of fifteen larval Habronema microstoma, including two

larvae similar in all respects to the one just described and the parasite illustrated in Fig. 29.

The larva illustrated in Fig. 29 was relatively much longer and narrower than that shown in Figs. 27 and 28, but there was otherwise little marked developmental change. The worm figured (Fig. 29), measured about 0.435 mm. long by 0.04 mm. wide at the base of the oesophagus and at the rectum. Scattered nuclei were seen in the oesophagus, intestine and body wall, those in the anterior portion of the body being few and scattered. At about 0.07 mm. from the anterior end there was some indication of a nerve ring. The base of the oesophagus was 0.165 mm. from the anterior end of the body, and at its base there was a slight dilation of the intestine. The anus was similar to earlier stages, but the tail was distinctly rounded. This larva is comparable with the stage of H. muscae represented in Fig. 9; the latter, however, was 0.115 mm. longer than the present specimen of H. microstoma.

The larva shown in Figure 30, although somewhat shorter than the preceding one, was evidently in a slightly more advanced stage of development. It measured 0.34 mm. long by 0.056 mm. wide at the rectum. The base of the oesophagus was 0.2 mm. from the anterior end, at which point the body was 0.05 mm. in diameter. The tip of the tail was 0.046 mm. from the anus. This larva was one of thirty or more found on January 4th, in the head of a Stomoxys pupa from the same culture as the two preceding ones (Experiment No. 12, page 26).

The next stage known to the writer is shown in Fig. 31. The worm figured was one of 35 larvae found in a Stomoxys fly (January 10th) from the same culture as the preceding stages (i.e., Experiment No. 12, p. 31). It measured 0.95 mm. long by 0.04 mm. wide at the base of the oesophagus. The base of the pharnyx and oesophagus were respectively 0.03 mm. and 0.38 mm. from the anterior end of the body. The anterior end of the oesophagus was about 0.01 mm. in diameter, increasing at the base to about 0.016 mm. At about 0.092 mm. from the anterior end there was a conspicuous nerve ring followed by a group of large nuclei. The anus, still closed, was about 0.043 mm. from the tip of the tail. The moulting cuticle completely enveloped the worm. This parasite was twenty days old, not more than seventeen days of which period may have been passed within the body of the fly, and it most resembled that stage of H. muscae illustrated by Fig. 14; the latter measured, however, 0.485 mm. longer than the former, but was apparently less developed. As previously stated (page 25), it was

seventeen days old, and had lived in the body of the fly not more than eleven days.

After undergoing at least another moult the minute spines at the tip of the tail are seen under the cuticle. The parasite now measures from 1.419 mm. to 1.550 mm. in length. This stage, which is comparable with Ransom's 5th stage of Habronema muscae, is generally found in the head or abdomen of pupae in an advanced stage of development.

Numerous larval parasites in this stage of development were found in a pupa on January 29th, in a culture (Experiment No. 13, page 32), which produced also the parasites of Figs. 21, 22 and 23. Reference to this experiment will show that the larvae were fifteen days old, and that not more than thirteen days of this period could have been lived within the body of the developing fly. A typical example measured 1.485 mm. long by 0.049 mm. wide at the base of the oesophagus, i.e., about 0.68 mm. from the anterior end, and about 0.033 mm. wide at the anus.

The oesophagus was 0.013 mm. in diameter at the anterior end, and increased to 0.030 mm. in diameter at its base. The nerve ring was about 0.1 mm. from the anterior end and the closed anus about 0.059 mm. from the tip of the tail. The moulting cuticle adhered somewhat closely to the body.

After this moult is completed, the spines are evident at the tip of tail, and the anus is no longer closed by the rounded projection seen in earlier stages. Fig. 32 shows the anterior end of a worm in this stage of development, which was found in the proboscis of a Stomoxys fly on January 12th. The parasite was reared from the same culture as those shown in the three preceding figures (Experiment No. 12), and was therefore twenty-two days old, not more than nineteen days of which period may have been spent within the intermediate host. It measured 1.560 mm. long by 0.0495 mm. wide at the base of the oesophagus, and 0.0297 mm. wide at the anal opening. The length of the pharynx was about 0.049 mm. The nerve ring and base of the oesophagus were respectively 0.1 mm. and 0.69 mm. from the anterior end. The oesophagus was 0.0135 mm. in diameter at its base. The anterior one-sixth was of fairly uniform diameter, but about 0.11 mm. from its commencement a marked increase in thickness occurred which was maintained through its remaining length.

The tip of the tail, which was about 0.069 mm. from the anal opening, was bluntly rounded, and ornamented with minute spines.

This is apparently the final larval stage of H. microstoma in the body of the fly, and the stage comparable with Ransom's 6th stage of H. muscae. Although larger larvae are found in flies, no further developmental changes, other than a general increase in size, have been observed in them. It is presumed, therefore, that in this stage the parasite is ingested by the horse, and thereafter continues its development in the stomach of the definitive host.

Fig. 33 illustrates the posterior end of a parasite found on the same day and in the same culture as the larvae shown in Fig. 32. Although the former was somewhat larger (see Table 2, Specimen 5), both were evidently in the same stage of development.

The maximum length known to the writer to be attained by the larval worm within the fly is 1.815 mm. (see Table 2, Specimen 1). A larvae of this length was found on January 16th in a fly which emerged two days previously from the same culture as the two last mentioned parasites, i.e., Experiment No. 12. Other larvae from the same fly measured from 1.584 mm. to 1.600.

The final larval stage of H. microstoma in the body of the Stomoxys fly measures from 1.570 mm. to 1.815 mm. long, and although considerably shorter than the final larval stage of H. muscae in the body of Musca domestica, it is in the same state of advancement as the latter, as shown, e.g., by the presence of the characteristic spinous-tipped tail.

Excepting only the earliest stage in the body of the fly-larvae (c.f. Fig. 20), all the larval stages of H. microstoma known to the writer occur sometimes in cysts.

Summary and Discussion.

From an analysis of the experiments carried out to determine the relationship of Musca domestica and Stomoxys calcitrans to the embryos of H. microstoma, it will be seen that Musca domestica exclusively was used in five experiments. In two cases no infection took place, while in the remaining three cases a total of only three individuals became infected, each with a single parasite. In all, 419 Musca domestica larvae, pupae, and flies were examined, with the result stated. In one of these experiments Musca domestica larvae failed to become infected in a culture, which subsequently infected 100% of the Stomoxys calcitrans examined; certainly the two species of fly larvae were at different ages, but other experiments with larvae of the same age of the two species together, would lead one to believe that the age of the fly larvae had no influence at

all on the result, as the worm larvae were actively alive. Stomoxys calcitrans exclusively was used in one experiment, with the result that 100% of the individuals subsequently examined were found to be more or less heavily infected. In another experiment both species of fly larvae were introduced at the same time to a culture of H. microstoma, with the result that 100% of the Stomoxys were heavily infected, whilst the Musca domestica were negative. In all, 71 Stomoxys larvae, pupae and adult flies were examined, of which number 48 were infected.

In the other two experiments (Nos. 15 and 16), a double culture, i.e., of Habronema muscae and of Habronema microstoma, was given as food to Musca domestica and Stomoxys calcitrans, in the same cage. Both of these experiments show undoubtedly that while Habronema muscae only occurs in Musca domestica, even when Stomoxys calcitrans is in the presence of an intense infection, Habronema microstoma occurs almost entirely in Stomoxys calcitrans, but also rarely in Musca domestica.

Upon the results of these experiments, supported by the finding of what was almost certainly a larval H. microstoma in a naturally infected Stomoxys fly (c.f. page 32), and further supported by the results of the experiments recorded on pages 16. 17, 18, 31 and 32 of this Report, the writer bases his conclusion that Stomoxys calcitrans is the principal intermediary host of H. microstoma, and that Musca domestica only occasionally (possibly only accidentally) acts as an intermediary.

In arriving at the first conclusion the writer has not lost sight of the fact that deposits of fresh horse faeces are not the usual breeding place of Stomoxys calcitrans. It is well known that this fly breeds frequently, if not generally, in decaying grass, straw, and similar matter, and also in loose soil contaminated by stable drainage. The larvae and pupae are to be found commonly, however, in the older portions of manure heaps, and in such situations as are to be found in crowded horse yards. It has been proved during these investigations, that under certain conditions, embryonic H. microstoma remain infective in faeces for a period of at least fifteen days.

The fact that fresh faeces are not usually used as a breeding ground by Stomoxys calcitrans offers no obstacle to Stomoxys calcitrans acting as a natural intermediary host of H. microstoma; rather, this with other facts mentioned above, suggests the only feasible explanation of some phenomena in the life of this form which do not appear to be otherwise explainable, namely, the

lengthy period of viability of the embryo, the relative scarcity in horses' stomachs of the adult stages of H. microstoma as compared with H. muscae and the apparent seasonal occurrence of the former species, from my observations, confined to a period from September to January.

Unlike the adult Musca domestica flies, which are to be found here more or less plentifully throughout the year, Stomoxys flies are extremely scarce during the winter and spring. These flies generally appear in numbers during the latter part of December, becomes increasingly numerous in January, February and March, and gradually disappear until very few remain in June. Why none of this species (H. microstoma) was found in the animals post-mortemed during late January to early March is not easy of explanation, since the fly host has been numerous, and the season very mild, and the horses examined came from various locations.

This investigation shows that the life-history of H. microstoma is somewhat similar to that of H. muscae, although the principal intermediary host is a different species of fly. Briefly summarised, the life-history is as follows :—The embryos passed out in the faeces from the horse are taken up by the larvae of Stomoxys calcitrans which have oviposited on the faeces. The faeces remain infective in this respect up to at least fifteen days, the fly-larvae being known to react to infection when two days up to nine days old, and possibly earlier and later. After apparently undergoing a slight development in the faeces (Fig. 19), the embryo of H. microstoma (Fig. 20) enters the larva of Stomoxys calcitrans (or rarely and possibly accidentally the larva of Musca domestica), where it continues to develop through the stages shown in Figs. 21-23.

Development continues in the pupa through the stages shown in Figs. 24-30; and in the adult fly, as seen in Figs. 31-33, in which condition it is ready to develop in the stomach of the horse, where it reaches maturity. Doubtless, infection of the alimentary canal of the horse is brought about at least in part by ingestion of living and dead infected flies.

Whether a living Stomoxys infected with H. microstoma is able to infect the definitive host with this parasite by means of direct inoculation into the skin yet remains to be proved. So far as my experiments go it has not done so, though this may have been due to a clogging of the proboscis by the fifteen and twenty parasites present in it in the two cases specially examined, preventing it from properly piercing the skin, over-infection thus defeating the object of infection of the intermediary.

C.—Habronema megastoma (Rudolphi, 1819).

1.—Historical.

As stated in the Introduction to Part I. of this report nothing definite appears to have been recorded of the life-history of H. megastoma, either in Australia or elsewhere.

Apparently (Railliet, 1916, p. 102) some reference is made to the larvae of this worm by Ercolani, 1859, but the paper has not been available to me for study.

Railliet (1895, p. 535) states that the life-history of this species is unknown, but that there is reason to suppose that the intermediate host is an insect found in fodder. The same author (page 534) states further that Chabert in 1782 recorded H. megastoma as the causative agent of tumours in the stomach of the horse.

2.—The Adult.

The adult of H. megastoma is easily separated from its congeners, H. muscae and H. microstoma, by its smaller size, and the very distinctive form of the anterior end. The worm, however, is so well known that it is quite unnecessary here to describe it in detail.

It would appear from the writer's observations that the adult stages of H. megastoma occur naturally only in tumours in the definitive host and that their rare occurrence on the external surface of the tumour or adjacent membrane is due to their escape from their natural surroundings after the death of the host. The occurrence within the tumours of young larvae, i.e., those in the final stage of development attained in the body of the fly will be referred to later on.

A census of the nematode parasites found in thirty-nine horses' stomachs examined during these investigations shows that nineteen stomachs contained well-developed tumours of H. megastoma (c.f. Table 9).

The adults are also known to occur in the splenic abscesses which appear to have become more common of recent years, and to be responsible for a considerable increase of mortality in horses in certain seasons and certain districts in South-Eastern Australia.

3.—Record of Experiments.

The technique employed in these experiments has been fully described on pages 12-15.

In the whole thirty-nine horses' stomachs examined, of which nineteen contained tumours of H. megastoma, I have never been able to find any stage, adult or otherwise, of H. muscae or H. microstoma associated with such tumours—which is in harmony with the commonly accepted statement that these tumours are due to H. megastoma. For this reason, therefore, it has been considered perfectly safe for experimental purposes to use the contents of such tumours to infect sterile faeces with the eggs and embryos of H. megastoma.

A. To determine the intermediary host or hosts of H. megastoma and the early life-history of the parasite.

Experiment No. 17.

On October 25th the contents of a stomach tumour or nodule containing embryos were mixed with sterilized normal saline and sterilized faeces, and without a preliminary period of incubation fed to four-days-old larvae of Musca domestica. All seventeen of the resulting adult flies were examined on November 6th, 7th and 8th, of which number six contained from one parasite in one fly to eight parasites in three other flies. These parasites measured from 0.21 mm. to 0.95 mm. long.

Experiment No. 18.

A similar experiment to No. 1 was commenced on November 26th with three-days-old Musca domestica larvae. Three days later a portion of the faecal matter was examined, and found to contain a few living embryos. Subsequently (November 29th to December 10th), the resulting larvae, pupae and flies, fifty-two in all, were examined with negative results.

Experiment No. 19.

The contents of a stomach tumour were mixed with saline and sterilized faeces on December 3rd, and incubated at a temperature of 22°C.—27°C. for seven days. On December 10th and subsequently, five-days-old larvae of Musca domestica were fed on the infected matter. On December 18th living embryos were still found in the faeces. Between December 17th and 21st, 8 larvae, 5 pupae and 77 adult flies were examined, of which number 3 larvae and 42 flies were found to contain larval parasites. One or two parasites, measuring 0.32 mm.-0.5 mm. long, were found in most of the flies, but in one case, ten parasites measuring about 0.86 mm. long, were found, eight of which were encysted.

Experiment No. 20.

This culture was prepared on December 13th in the same manner as the three preceding ones. Musca domestica larvae (three days old) were fed upon the infected matter from the time of its preparation (December 13th) until they pupated, or were dissected. On December 21st and five succeeding days a total of 36 larvae, 15 pupae, and 33 adult flies were examined, of which number 13 flies only were found to be infected with from 1 to 16 larval parasites each. About 50% of the larval parasites were encysted.

Experiment No. 21.

This culture was prepared on December 19th in the same manner as the preceding ones. On the same day the infected material was given as food to "clean," two-days-old larvae of Stomoxys calcitrans, and on January 4th and 5th 55 adult flies resulted, and were examined, with negative results.

Experiment No. 22.

Embryos from thirty or more gravid H. megastoma were mixed with saline and sterile faeces on January 23rd, and incubated at room temperature until January 29th, on which date living embryos were found in culture. Five-days-old larvae of Stomoxys calcitrans were fed upon the infected matter on January 29th and subsequent days, and on February 14th 27 of the resulting adult flies were examined, none of which was infected.

B. To determine the frequency of larval Habronema megastoma in Musca domestica and their abundance and location in the body of the host.

(i.) Free or caught flies.—Larval H. megastoma have not been identified in naturally bred flies that have been caught.

(ii.) Laboratory bred flies showed a lower percentage of infection than with either H. muscae or H. microstoma, as will be seen by reference to Experiments No. 17, page 47, No. 19, page 47, and No. 20, page 48.

In the first of these experiments (No. 17) about 35% of the flies examined were infected; the number of parasites found in each Musca domestica fly varied from 1-8, the average being two. The head and thorax were the only regions of the body infected.

In the second positive experiment (No. 19), 50% of the larvae and flies examined were infected, the number of parasites in each varying from one to ten. No embryos could be found in the pupae. The abdomen only of adult flies was infected, and in one of these eight of the total number of ten parasites were found in cysts.

In the third positive experiment (No. 20), about 15.5% of the flies (adults only) were infected with from one to sixteen parasites each, all of which were located in the abdomen only. Of the total number of parasites from this culture about 50 per cent. were encysted.

The negative results obtained in Experiment No. 18 are very strange in view of the fact that the only definite difference between this and Experiment No. 17, which was distinctly positive, was in the younger age (three days) of the fly larvae used in Experiment No. 18, and in the fact that examination of the larvae, pupae, and flies was commenced at three days instead of eleven days, though in both cases it was continued up to fourteen days. The fact, however, that only a few living worm embryos were found in the culture three days after the commencement of the experiment suggests that either the infestation of the culture was not sufficiently massive, or that some unknown occurrence had killed off the majority of the worm embryos.

C. To determine the viability of embryonic Habronema megastoma in faeces.

Experiment No. 19, page 45, shows that embryonic H. megastoma may survive for a period of at least fifteen days in sterilized faeces and remain infective to larvae of Musca domestica for a period of at least seven days. Further, if one may judge by analogy with H. microstoma the survival of H. megastoma larvae for probably at least fifteen days in the sterile faeces suggests that such infected faeces may remain infective for the full fifteen days. At the end of this fifteen days, during which the embryos in this culture were incubated at a temperature of 22°C., 27°C., they were found to be in the condition shown in Fig. 38, a and b.

Reference to experiments with embryonic H. muscae and H. microstoma shows that the periods of viability in these species was not less than eight (the longest period tested) and fifteen days respectively.

D. To determine the period of survival of larvae removed from flies.

At 4. p.m. on December 21st, six of the encysted H. megastoma larvae referred to under Experiment 19, page 47, were placed in sterilized normal saline, contained in a covered Petri dish. All were living on the following afternoon, three died before 10 a.m. of the next morning, December 23rd (forty-two hours), two died before 10.30 a.m. on the next day (December 24th), and the last died before 4.30 on that afternoon, a maximum of four days. These parasites were in the stage of development shown in Fig. 45.

On December 24th (noon), eleven larval H. megastoma, from Experiment No. 20, page 48, were treated as above. Four died before noon on December 26th (two days), four before noon on December 27th, and the remaining three before noon on December 28th (maximum of four days). These parasites were about 1.6 mm. long, and therefore probably in the stage of development shown in Fig. 46.

These experiments are, of course, quite insufficient to give any positive evidence, but in each case the larvae would appear to live much longer than do those of H. muscae and H. microstoma under similar conditions, suggesting a much greater resistance on the part of the H. megastoma embryos than that of the other species.

E. To determine the period of survival of larval H. megastoma in dead flies.

Three flies from Experiment No. 20, page 48, which emerged on the afternoon of December 23rd, were killed in a cyanide of potassium killing-bottle on the morning of December 24th, and placed on moist filter paper in a covered dish. One fly was dissected late in the afternoon, and found to contain two living parasites. The others were examined early on December 26th, when one only was found to be infected, the two parasites which were contained in the abdomen being dead.

4.– Development.

The eggs and young embryos of Habronema megastoma, as found in the uterus of the gravid worm, are illustrated in Fig. 36. The egg (Fig. 36a) measures from 0.04 mm. to 0.05 mm. long by about 0.01 mm. wide, and is similar to that of H. muscae. Later stages in development are shown in Fig. 36b, and c, which measured 0.05 mm. long by 0.013 mm. wide, and 0.053 mm. long by 0.015

mm. wide respectively. Fig. 36d shows the young embryo in a condition sufficiently far advanced to enable it to be definitely distinguished from embryos of H. muscae and H. microstoma by the relative position of the anterior to the posterior end. As will be seen on comparing Fig. 1, of H. muscae, Fig. 18, of H. microstoma, and Fig. 36, of H. megastoma, the tail of the young embryo in the first species never reaches more than half-way along the main part of the body when bent in the egg. In H. microstoma, though a stage comparable with Fig. 1e is not shown, it does occur. In H. megastoma, however, the embryo is seen to be bent in about the middle of its length in several stages. Thus the parasite as it leaves the parent worm, as well as in some subsequent stages (as will be seen later) contrasts in a marked degree with H. muscae and H. microstoma.

In the stage shown in Fig. 36d, the embryo measured 0.056 mm. long by 0.0165 mm. wide.

Still further development is seen in Fig. 36e, which measured 0.056 mm. long by 0.018 mm. wide. Fig. 36f shows an embryo 0.057 mm. long by 0.017 mm. wide in the most advanced stage attained in the uterus of the parent worm. As is the case with the other species dealt with in this report the form of anterior end can be made out only with difficulty. At the anterior end there is evidence of a horn-like process, and a rudimentary pharynx with an adjacent clear space as in H. muscae, but the more or less conspicuous nuclei observed in H. muscae and H. microstoma have not been observed in such young embryos of this species.

Development of the embryo in the tumour.

Further development takes place during the life of the embryo in the purulent matter contained in the stomach tumour. The embryos illustrated in Fig. 37 were found in such circumstances as were others which had ruptured the egg-shell or sheath.

Development of the embryo in faeces.

Doubtless the embryo is carried into the stomach of the horse with the purulent discharge from the several openings in the summit of the tumour, but embryos have not been identified in the stomach or intestines nor in the faeces of naturally infected horses.

The development which probably takes place in the faeces, is illustrated in Fig. 38, a and b. These embryos, taken from an adult worm, were incubated in sterilized faeces for a period of

fifteen days at a temperature of 22°-27° C. (c.f. Experiment No. 19, page 47). The parasite shown in Fig. 38a, which was not enclosed in a sheath, measured about 0.0528 mm. long from the bend in approximately the middle of the body to the anterior end, by about 0.0065 mm. in diameter at its widest part. A large nucleus occurred at about 0.043 mm. from the anterior end, and a smaller one was seen posterior to the first. In some specimens one large nucleus only was seen, and in others there were one or two nuclei as above, and one smaller nucleus at about 0.039 mm. from the posterior end. Other embryos enclosed in sheath or egg-shell were found on the same day and in the same culture. The embryo shown in Fig. 38b measured about 0.104 mm. long from the anterior end to the tip of the tail. The body was bent in the middle in the characteristic manner, and the large nucleus was somewhat further back than usual, i.e., 0.053 mm. from the anterior end. Posterior to what was evidently the rudimentary pharynx there were two clear spaces, which were also observed in the specimens shown in Fig. 36f and Fig. 38a. Posterior to these clear spaces and in the middle line of the anterior fourth of the body was a noticeable aggregation of minute granular bodies, probably the earliest evidence of the developing alimentary system.

Development in the larvae of M. domestica.

Early larval stages of H. megastoma comparable with the stages of H. microstoma illustrated by Figs. 20, 21, 22, and 23, have not been found. The earliest stage known to the writer to occur in the intermediate host is shown in Fig. 39. This parasite was found in a fly larva on December 21st from a culture of sterilized faeces, which was infected on December 3rd, with the contents of a stomach tumour, and then incubated for a period of seven days before being exposed to ingestion by the fly larvae than five days old (c.f. Experiment No. 19, page 47). The parasite was therefore eighteen days old, eleven days of which period may have been passed in the body of the fly larvae. The true period was, however, probably much shorter (see text referring to Fig. 43). The length of the worm was 0.221 mm., and the width near the rectum about 0.046 mm. The base of the oesophagus was 0.099 mm. from the anterior end of the body, and the tip of the tail about 0.042 mm. from the anal operculum.

Two other larvae from the same culture, examined on the same day as the above, were each found to contain one parasite measur-

ing 0.21 mm. and 0.25 mm. long respectively. Both were apparently in the same stage of development.

Had the fly larvae in this culture developed at their usual rate of progress, they should have been well advanced pupae instead of larvae on December 21st. Presumably, therefore, the larval H. megastoma should ordinarily attain the stage of development shown in Fig. 39, whilst its intermediary host is in the pupal stage. Although older and shorter, the larval parasites just referred to were considered to be in a stage of development most comparable with the H. muscae larva shown in Fig. 6.

It is assumed from the examination of a parasite which was found on November 6th in an adult fly from Experiment No. 17, that an intervening moult takes place before the parasite reaches the stage figured in Fig. 40. The larva measured 0.28 mm. long by about 0.04 mm. wide near the rectum, and about 0.038 mm. wide at the base of the oesophagus, which was 0.115 mm. from the anterior end of the body. The moulting cuticle at the anterior end of the body appeared as shown in Fig. 40, which illustrates a larva found in a fly on the same day and from the same culture. Unfortunately the specimen referred to was lost before a drawing and further details could be secured. As will be seen by reference to Experiment No. 17, this parasite was twelve days old. The whole of this period may have been passed within the body of the intermediate host, but this is very improbable, as will be seen in the following paragraph.

The parasite shown in Fig. 40 was found, as already stated, on November 6th in an adult fly from the same culture as the parasites shown in the preceding paragraph; it was therefore twelve days old, and may have passed that length of time in the intermediary host. This larva measured 0.33 mm. long by about 0.038 mm. wide at the base of the oesophagus, which was 0.128 mm. from the anterior end of the body. The pharynx was 0.01 mm. in length, and surrounded at its base by a clear space or vacuole. The oesophagus was 0.012 mm. in diameter at the anterior end, and somewhat larger near the base. Numerous moderately large nuclei were seen in the whole length of the alimentary tract. A group of similar nuclei occurred also at about 0.065 mm. from the anterior end of the body, and elsewhere in the body wall, as shown in Fig. 40. The worm had evidently nearly completed a moult.

The larvae illustrated in Fig. 41 and Fig. 42, also taken from an adult fly, had the same history as the one shown in Fig. 40. The former measured 0.36 mm. long by about 0.040 mm. wide at the

base of the oesophagus, i.e., at 0.12 mm. from the anterior end of the body. Another worm of the same total length and from the same fly measured 0.145 mm. from the anterior end of the body to the base of the oesophagus. The worm shown in Fig. 42 was 0.37 mm. long by about 0.040 mm. wide at the base of the oesophagus, which was 0.14 mm. from the anterior end of the body. In these three worms and most others in a similar stage of development the cuticle at the anterior end was somewhat separated from the body, as seen in Figs. 41 and 42. The nuclei in the alimentary tract were fewer and smaller than those seen in the larva illustrated in Fig. 40, apparently indicating that the latter was hardly so far advanced, since these nuclei are at their maximum almost if not quite as soon as they are first noticeable. Evidently this stage (c.f. Figs. 41 and 42) is comparable with Ransom's Stage 1 of H. muscae and also with the eight-day-old larva (H. muscae) shown in Fig. 8 of this Report, both of which were from fly pupae.

The parasite illustrated in Fig. 43 was evidently in a more advanced stage than the larvae illustrated in Fig. 41 and Fig. 42. It was found on November 8th in a fly from the same culture as the latter, and was, therefore, fourteen days old. The body was 0.5 mm. long by about 0.04 mm. wide at the base of the oesophagus, which was about 0.17 mm. from the anterior end of the body. The pharynx, which was about to moult, was about 0.015 mm. long, and a clear space surrounded it. Nuclei were seen in the body wall and alimentary tract, but they were not made out in that part of the body where, in later stages, the nerve ring is to be found. This stage is near that stage of H. muscae designated by Ransom as Stage 2.

The next stage in development of the larva known to the writer is shown in Fig. 44. The parasite was found on December 20th in an adult fly from the same culture as the parasite shown in Fig. 39, which was found in a fly-larva on December 21st (c.f. p. 52) and also Experiment No. 19, page 47.) The latter (Fig. 39), therefore, may have lived one day longer in the intermediate host than the former (Fig. 44), but as it was considerably less developed it may be assumed that its life in the intermediary was at least several days less than that of the more developed specimen. The larva (Fig. 44) measured 0.87 mm. long by 0.045 mm. wide near the base of the oesophagus. At the base of the pharynx, which was about 0.016 mm. from the anterior end, the body was 0.029 mm. in diameter, increasing to about 0.06 mm. at the base of the oesophagus. The oesophagus measured 0.013 mm. in diameter at

the anterior end, and increased to about 0.02 mm. at its base, which was about 0.247 mm. from the anterior end of the body. The anus was evidently still closed as in early stages. The nerve ring is now clearly distinguishable, being about 0.073 mm. from the anterior end. A few nuclei were seen near the nerve-ring, and in the body wall. The pharynx and posterior end were moulting.

Other larval parasites, including specimens 4-9, of Table 3, were found in flies from the same culture and on the same day. These parasites measured from 0.98 mm. long in Specimen No. 9, to 1.280 mm. long in Specimen No. 4. Possibly all of this series are merely successive steps in the progress of larval development from one definite stage (c.f. Fig. 44) to another (c.f. Fig. 46). All of these most closely resemble the stage of H. microstoma represented by Fig. 14, and therefore Ransom's Stage 3 of H. muscae.

One of the series of larvae referred to above (Specimen No. 7, Table 3) is shown in Fig. 45. In this specimen the cuticle at the anterior end of the body was transversely ribbed and oral lips were seen distinctly. These two features were not observed in less developed specimens. The anus was closed as in earlier stages.

The parasite shown in Fig. 46 (c.f. Table No. 3, Specimen No. 3) is in a markedly more advanced stage of development than any of the preceding ones. Two very definite, and for the purpose of diagnosis very important, characters manifest themselves in the worm, namely:—(1) The oral cavity is distinctly widened anteriorly, thus simulating the characteristic funnel-shaped anterior end of the alimentary canal of the adult, which character alone is sufficient to distinguish this larval stage of H. megastoma from any stage of either H. muscae or H. microstoma (in both of which species the oral cavity is not so widened); (2) a constriction of the body near the anterior end like that so conspicuous in the adult, is distinctly seen beneath the cuticle of the worm.

Whether or not a moult occurs just before these developments become visible is not known. This parasite (Fig. 46) was found on December 26th, encysted in the abdomen of an adult fly, from Experiment No. 20. It will be seen, therefore, that this stage in the development of H. megastoma can only be compared with the corresponding stages in H. muscae (c.f. Ransom's 4th Stage), and in H. microstoma (my Fig. 31) in that this forms an intermediate condition between Stages 3 and 5, recognised as being comparable in other respects in all three species.

The embryo from which it was reared was obtained with many others, from a stomach tumour on December 13th, and on the same

day the culture was offered as food to three-days-old larvae of H. domestica. It was therefore thirteen days old, the whole of which period may have been passed within the body of the intermediary host. The worm was 1.650 mm. long by about 0.033 mm. wide at the base of the pharynx, and 0.053 mm. wide at the base of the oesophagus. The base of the pharynx and oesophagus were about 0.033 mm. and 0.428 mm. respectively from the anterior end of the body. The anal opening, which was 0.069 mm. from the tip of the tail, was still closed as in earlier stages.

The next stage in the development of the larval parasite is illustrated by Fig. 47 (c.f. Table No. 3, Specimen No. 2). The specimen here figured was 1.848 mm. long by 0.082 mm. wide at the base of the oesophagus, ie., at 0.56 mm. from the anterior end of the body. The base of the pharynx and the nerve ring were 0.04 mm. and 0.132 mm. respectively from the anterior end of the body. The anus was still closed, minute spines were evident beneath the cuticle at the tip of the tail, indicating that this stage is comparable with the larval stage of H. microstoma referred to on page 42 of this Report, and with Ransom's 5th stage of H. muscae. This larva was encysted in the abdomen of a fly found on the same day and in the same culture as the parasite in Fig. 46 (c.f. Experiment No. 20, p. 48); it was therefore thirteen days old, and may have lived for the whole of that time in the body of the intermediary fly. It was about to moult.

The final larval stage in the body of the intermediate host is seen in Fig. 48 and Fig. 48A (c.f. Experiment No. 20, page 48). The history of this larva was the same as that of the larva shown in Figs. 46 and 47; it was therefore thirteen days old. As will be seen, the limit of possible age of the larvae in Figs. 46 and 47, and in 48 and 48A is the same, but there is no doubt in my mind, from the internal evidence of the stage of development reached by these two lots of larvae, that those seen in Figs. 46 and 47 were not ingested by the fly larvae so early as was that seen in Figs. 48 and 48A, the latter being now undoubtedly further advanced, and therefore obviously older than the former. It measured 2.244 mm. long by 0.096 mm. wide at the base of the oesophagus, which was 0.63 mm. from the anterior end of the body. The base of the pharynx and the nerve ring were about 0.066 mm. and 0.138 mm. respectively from the anterior end of the body. (For other measurements see Table No. 3, Specimen No. 1). The anus, closed in all earlier stages, was open and the tip of the tail was spinous (c.f. Fig. 48A).

The parasite was in a stage comparable therefore with the larval

H. microstoma shown in Fig. 32, and with that stage of H. muscae designated by Ransom, Stage No. 6.

This final larval stage of H. megastoma found in the body of the adult fly on one occasion only, has also been found frequently, in company with adults, within stomach tumours. Measurements and details of a number of examples of this final stage are given in Table 4.

Thus between the very early stages represented by embryos such as that shown in Fig. 37, and very advanced stages such as shown in Figs. 48 and 48A, both of which were recovered from the interior of tumours, there is a considerable number of stages of development never yet found in the stomach tumours or in the digestive canal of the host, but which form a continuous series of progressive steps, almost entirely (Figs. 39-47) passed in the body of the larva, pupa and imago of Musca domestica, the final stage (Figs. 48 and 48A), being found both in Musca domestica (adult), and in the contents of the stomach tumours of the horse, where they develop into the adult worm.

Summary and Discussion.

It will be seen from an analysis of the experiments carried out to determine the relationship of Musca domestica and Stomoxys calcitrans to the embryo of H. megastoma that Musca domestica exclusively was used in four experiments, and that infection took place in three of these experiments. The failure to infect flies in the fourth experiment cannot be accounted for.

In the positive experiments 35%, 52%, and 15% respectively of the larvae, pupae and adults examined, were infected with from one to sixteen parasites each.

Stomoxys calcitrans exclusively was used in two experiments, in both of which there was no infection.

It was intended to carry out further experiments, viz. :—(1) A culture of embryos of H. microstoma and H. megastoma fed to larvae of both species, and (2) a culture of embryos of all three species of Habronema fed to larvae of both species of fly. Time however did not permit of this being done, nor, chiefly for this same reason, was it found practicable to experiment with other species of Musca.

These experiments show that (1) Musca domestica is an intermediary host of H. megastoma, and (2) all the available evidence is against Stomoxys calcitrans acting, even accidentally, in such

capacity. Thus embryos passed out in the faeces from the horse are taken up by the larvae of Muscae domesctica adults, which have oviposited on the faeces. The faeces remain infective to the fly larvae in this respect up to at least fifteen days after leaving the rectum.

Fly larvae are known to react to infection when three days up to five days old, and possibly earlier and later. After a certain amount of development in the faeces (c.f. Fig. 38) the embryo of Habronema megastoma enters the larva of Musca domestica, then it continues to develop through the fly pupa and adult fly as seen in Figs. 40 to 48, in which condition it is ready to complete its development in the stomach of the horse; where larvae of such a stage have been met with (c.f. Figs. 49 and 49A, and also Table No. 4). H. megastoma has not been found in any of the 182 adult Musca domestica and 63 adult Stomoxys calcitrans caught in the stables during the period May-November, 1917.

In its three last stages in the body of the fly H. megastoma may be differentiated from both H. musca and H. microstoma in corresponding stages of development.

General Summary, Comparison, and Discussion.

Intermediary host.

It has been shown that each of these three species of Habronema found adult in the horse, H. muscae, H. megastoma and H. microstoma require for the completion of their life-cycle that they shall enter the body of a fly; in the case of H. muscae, Musca domestica; in H. megastoma also Musca domestica; and in H. microstoma, Stomoxys calcitrans, or very rarely under experimental conditions, Musca domestica, i.e., H. muscae and H. megastoma find their intermediary host in a non-piercing sucking fly, as distinguished from H. microstoma, which finds its chief host (at least) in a piercing sucking fly. The possible importance of this distinction will be referred to a little later.

It is, of course, quite possible that species of flies other than those experimented with, as herein recorded, are involved in the carriage of these Helminth parasites, but time and availability of material has not so far allowed of experimentation with them. For example, Musca australis, which in the writer's opinion, is likely to be a frequent carrier of Helminth infection, has not been experimented with, "clean" flies not being available at the time the helminth material was at hand. In this connection it is of interest to note

that Johnston (1912, p. 76) has recorded a larval nematode resembling Habronema from Musca vetustissima.

Methods of Infection of Flies.

From the experiments herein detailed, it is obvious, in the case of H. muscae and H. megastoma, since their intermediary host, M. domestica, breeds chiefly in manure heaps that eggs containing embryos or the very early larvae of these two helminths present in the faeces of the infected horse, are taken up during feeding by the larvae of the fly host, in which they continue their development.

In the case of H. microstoma and Stomoxys calcitrans this may seem to be a more difficult matter to understand since Stomoxys calcitrans, by reason of its piercing proboscis is apparently preeminently fitted for the carriage of those parasites which pass some stage in the blood-stream of the host whence the adult fly derives its food supply. We have no reason, however, to suppose either that Habronema larvae ever occur in the blood-stream, or if they do, that this would form their normal method of transmission. It is evident on the other hand, that since Stomoxys does breed at times in stable manure, the circumstances are propitious for the ingestion of the embryo-containing eggs or young larvae of H. microstoma from infected faeces by the larvae of Stomoxys calcitrans, as has herein been shown to occur, and that to a marked extent.

It has been shown during these investigations that faeces infected with the embryos of H. microstoma and H. megastoma remain infective to the larvae of Stomoxys calcitrans and Musca domestica respectively for periods up to fifteen days in each species and in the case of H. muscae, the embryos have been shown to be infective to larvae of Musca domestica for periods up to eight days, i.e., the largest period tested. It has been shown further that the larvae of both species of fly react to infection when from two days to nine days old.

No evidence is forthcoming to show that the helminth larvae can enter the fly-larvae by penetration or in any way other than by ingestion.

Development within the Flies.

Commencing from the earliest larval stage of each of the Helminths under discussion, found in the larvae of the respective fly-hosts, it has been found that the "six stages" shown by Ransom, and confirmed by my own experiments to occur in the life of H.

muscae within the intermediary, also occur in a closely similar manner in H. megastoma and in H. microstoma.

It is worth recalling here that I have been able to show that several steps in the development of H. muscae occur in the fly-larvae and pupae of Musca domestica before the stage in the fly-pupae which Ransom has designated Stage 1, but for the convenience of other workers, I have adhered to the designations adopted by Ransom, in spite of the criticisms made by Seurat.

In each case, the "fifth stage" is that in which the spinous character of the tip of the tail is first seen, viz. :—beneath the cuticle, the anal operculum being still present, and the anus closed. The "sixth stage" is characterised by the further development of the spinous tip of the tail, by the disappearance of the anal operculum and the opening of the anus.

In the preceding text it has been stated that it is possible to differentiate between H. megastoma on the one hand and H. muscae and H. microstoma on the other in the later larval stages reached in the body of the fly, for the characteristic appearance of the head of the adult H. megastoma can be detected in the fourth larval stage of this worm as found in the adult Musca domestica. Further, comparative measurements have been given to support the conclusion that it is possible also to differentiate between larval H. muscae and H. microstoma in at least the sixth or final stage. In view of the importance attached to the correct specific diagnosis of the parasites found in, and supposed to be the causative agents of, the lesions known here as "Habronemic conjunctivitis," and "Habronemic granulomata," and also to enable others to more correctly identify larvae belonging to any one of these three species found in caught flies of the same and other host species, it appears desirable to discuss further one aspect of the evidence on which the writer has based his statements and conclusions, namely, the evidence contained in Tables 1, 2, 3, and 4 of this Report. For this purpose the length of the body and the distance of the pharynx, nerve-ring and oesophagus respectively from the anterior end of the body will be considered. In Table I (H. muscae from "clean" adult Musca domestica), Specimens 1-6, all of which are in the sixth or final stage attained in the body of the fly, show the length of the body to be from 2.310 mm.-2.541 mm., an average length of 2.398 mm. The length of the oesophagus is from 0.83 mm.-0.97 mm., or an average length of 0.898 mm. The distance of the nerve-ring from the anterior end of the body is from 0.132 mm.-0.14 mm., an

average distance of 0.1353 mm.; the length of the pharynx is from 0.046 mm.-0.049 mm., or an average of 0.047 mm.

In Table 2 (H. microstoma from "clean" adult Stomoxys calcitrans), Specimens 1-6, all of which are in the same relative stage of development as the above, show the body to be 1.600 mm.-1.815 mm. long, the average being 1.731. The length of the oesophagus is 0.65 mm.-0.792 mm., or an average length of 0.7403 mm. The distance of the nerve-ring from the anterior end of the body is 0.105 mm. to 0.132 mm. long, or an average distance of 0.1194 mm. (in five specimens only). The length of the pharynx is 0.046 mm.-0.053 mm., or an average length of 0.0505 mm.

Table 3 (H. megastoma from "clean" adult Musca domestica) includes only one specimen in a stage comparable with the above, i.e., the sixth stage. This worm (Specimen No. 1) measured 2.805 mm. long. The oesophagus, nerve-ring and base of the pharynx were distant respectively 0.63 mm., 0.138 mm., and 0.066 mm. from the anterior end of the body.

In Table 4 (H. megastoma from tumours), Specimens 1-6, all of which are in the same stage of development as Specimen 1 in Table 3, show the length of the body to be 2.310 mm.-2.805 mm. long, or an average length of 2.678 mm. The length of the oesophagus is 0.58 mm.-0.75 mm., or an average length of 0.685 mm. The distance of the nerve-ring from the anterior end of the body is 0.148 mm.-0.181 mm., or an average distance of 0.164 mm. The pharynx varied in length from 0.079 mm.-0.089 mm. the average length being 0.083 mm.

The following tabulated statement shows the average length of the body and parts referred to of the above six specimens each of H. muscae, H. microstoma (from "clean" adult flies), and H. megastoma (from tumours) and of one H. megastoma from a "clean" adult fly. Unfortunately only one H. megastoma from a fly was available for measurement.

	H. muscae	H. microstoma	H. megastoma	H. megastoma
	Average of 6 "6th stage" larvae from adult M. domestica flies.	Average of 6 "6th stage" larvae from adult Stomoxys flies.	One "6th stage" larva from an adult M. domestica fly.	Average of 6 "6th stage" larvae from a stomach tumour.
	mm.	mm.	mm.	mm.
Length of body	2.398	1.731	2.244	2.678
Oesophagus, base from anterior end of body	.898	.740	.630	.685
Nerve Ring	.135	.119	.138	.164
Pharynx	.047	.050	.066	.083

Neither the excretory opening and its main tube, nor any indication of the developing genital organs has been seen in any of the larvae herein described.

Bull (MSS. 1918), who records having reared H. megastoma from the embryonic stage to the "sixth stage," in Musca domestica, considers that in the final larval stage reached in the body of the fly H. megastoma cannot be differentiated from larval H. muscae in the same relative stage of development. The evidence I have adduced does not support this conclusion. This investigator used a culture of H. megastoma (from a tumour) in non-sterilized faeces from a horse which was apparently not subsequently post-mortemed to determine the presence or absence of H. megastoma in the stomach. Previously, however, he bred flies from larvae fed on faeces from the horse, and finding them all negative for Habranema he assumed that the horse did not contain Habronema embryos. Some of my experiments, notably Experiment No. 18, page 47, show that flies may be reared from faeces known to be infected and yet, on examination, prove to be negative for Habronema. The possibility that Bull was dealing with H. muscae and not H. megastoma is therefore not excluded.

As to the path taken by these Helminth larvae within the fly from the time the larvae are ingested by the fly-larvae, the evidence is not as complete as could be desired. I can say, however, that with regard to H. muscae and H. microstoma, the youngest larvae of these species, found in the larvae of Musca domestica in the former case, and in larvae of Stomoxys calcitrans in the latter, occur in the alimentary canal.

The next succeeding stages in each case are found either free in the body cavity or encysted in the fat-body of the larvae. No young stages of H. megastoma corresponding to the above are yet known in the larvae of M. domestica. In the pupa the helminth larvae are still found free or encysted in the broken-down tissues.

The exact position taken up by the developing parasitic larvae in the tissues of the pupa and early imago have not yet been determined. In flies that have emerged from the pupal case, the parasites in the case of H. muscae and H. microstoma have been found free in the body cavity of the abdomen, thorax, and head of the fly-host, and also encysted in the tissues surrounding the alimentary canal and on the surface of the tracheae. This is also true of H. megastoma, except that the solitary example of the "6th stage" of this parasite found in the fly host (M. domestica) was free.

It is interesting to note that in Musca domestica, even when very heavily infected with H. muscae, I have never been able to find any larvae in the proboscis proper, i.e., not further forward than the rostrum. On the other hand, as already stated, the proboscis of Stomoxys calcitrans most frequently harbours the "Sixth Stage" larvae of H. microstoma.

Whether there is any tendency for the larvae to gravitate towards the head of the fly-host I cannot positively say, except that in the case of M. domestica the majority are usually in the abdomen, whereas in Stomxys at least half the total parasites found are in the head.

Method of Infection of the Horse.

In view of the present knowledge the two obvious possibilities are (1) ingestion of the infected fly, or of free larvae which have escaped from the fly-host into the food or drink of the horse, or of larvae which have escaped from the fly-host on the lips or spot accessible to the lips or tongue of the horse, and (2) by penetration of, or inoculation into, the skin of the horse.

It is somewhat curious that in all these experiments I have never once come across any flies in the stomach of the horse, although I have no doubt that they are ingested and so reach the stomach. I have therefore no positive evidence as to whether the larva of the worm is passively liberated by the digestion of the fly, or whether it escapes prior to such digestion. I am strongly inclined, however, to think that the former is the case.

As to the possible infection of the horse by way of the skin, the evidence is quite insufficient. It has been shown very clearly (Figs. 34 and 35) that the "sixth stage" or final larval stage of H. microstoma finds its way very freely into the piercing proboscis of the adult Stomoxys calcitrans, but whether under any circumstances this "sixth stage" larva of H. microstoma can be transmitted to the skin or blood-stream of the equine host is not known—the only experiments to this end so far attempted by the writer having failed apparently from too heavy infection of the fly-host, so choking the proboscis.

As shown by Descazeaux, Rivolta and others (Railliet's Report, 1915), and by Bull (1916), and Lewis and Seddon (in press) in Australia, the larvae of some member of the Superfamily Spiruroidea (characterised by the presence of a spinous tipped tail) are undoubtedly associated with the formation of lesions in the skin known as "Cutaneous habronemiasis," and "Habronemic conjunc-

tivitis,'' these larvae being regarded by Railliet and Bull as the larvae of an equine species of Habronema. The present writer cannot, however, from the material and evidence so far at his disposal definitely refer the larvae so far seen to anyone of the three species of Habronema, the development of which is herein described. It was at first thought when it was found that Stomoxys calcitrans was the chief intermediary host of H. microstoma, that Bull (1916) was right in so far as he believed these larvae found by him in such lesions were introduced by a biting insect and that really they were the larvae of H. microstoma. I am indebted to Lewis and Seddon for allowing me to examine their material, and in the only specimen in which the anterior end is in a sufficiently good condition for this purpose (their Specimen No. 3), the pharynx of the larva in the lesion is undoubtedly not that of H. muscae, nor apparently that of H. microstoma. It is just possible that it is that of a H. megastoma larva, but the other characteristic features of the head of a larva of H. megastoma of this stage of development are entirely wanting. So that, as stated above, I cannot regard it as in any way proved that the examples of "Habronemic conjunctivitis" under my notice have been caused by any of the three equine Habronema species.

Development into adult in Horse.

Once freed from the fly-host in the stomach of the final host, the "sixth stage" larva develops into the adult in the stomach contents in the case of H. muscae and H. microstoma; these are possibly kept from being carried away in the ingesta by inserting their heads into the mucous membrane, or at least into the lumen of the glands.

In the case of H. megastoma, the larva either finds its way into a nodule already formed (since, as already stated, the final larval stage has been frequently found with adults in fully formed tumours or nodules), or else penetrates the lumen of glands, there setting up the irritation which results in the formation of a new tumour. What determines which of the two shall occur I cannot say.

Development of next generation in stomach and intestine of final host and its faeces.

As already seen, the eggs containing embryos must be poured out in considerable numbers into the interior of the nodule in the case of H. megastoma, and thence into the cavity of the stomach,

and in H. muscae and H. microstoma directly into the stomach. As seen by comparing Figs. 1d and 4 of H. muscae and Figs. 18d and 20 of H. microstoma, the former in each case being that of embryos still within the egg-shell as extruded from the female and present in the stomach contents, the latter being the first stage obtained from a larva in each case, very little development goes on after emergence from the egg-shell, either in the stomach or intestines, or in the faeces, i.e., until the helminth larva enters its new intermediary host. In the case of H. megastoma the young embryo enclosed within the egg shell is seen in Fig. 36f, but unfortunately a young larva comparable with Fig. 4 of H. muscae and with Fig. 20 of H. microstoma has not been found in the case of H. megastoma.

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DESCRIPTION OF PLATES.

Plate II.—Habronema muscae.

Fig. 1.—Egg and embryos from female.
Fig. 2.—Embryos from faeces of horse.
Fig. 3.—Embryo after six days' incubation in faeces.
Fig. 4.—Embyro from a Musca domestica larva.
Figs. 5-12 (inclusive).—Larvae from Muscae domestica pupae.
Fig. 13.—Larva (anterior end) from a Musca domestica pupa.

Plate III.—H. muscae.

Fig. 13a.—The above (posterior end).
Fig 14.—Larva (anterior end) from Musca domestica fly.
Fig. 15.—Larva (anterior end), from Musca domestica fly.
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Fig. 17.—Proboscis of House Fly (Musca domestica), showing two larvae of Habronema muscae.

All Figures, excepting 17, outlined by camera lucida.

Figures 1-9 and 11-13a inclusive from life.

Figures 10, 14, 15 and 16 from specimens fixed in alcohol and mounted in glycerine jelly.

Fig. 17, Photomicrograph.

Plate IV.—H. microstoma.

Fig. 18.—Egg and embryos from female.
Fig. 19.—Embryo after five days' incubation in faeces.
Fig. 20.—Embryo from a Stomoxys calcitrans larva.
Figs. 21-23 (inclusive).—Larvae from Stomoxys calcitrans pupae.
Fig. 24.—Larva from Stomoxys calcitrans pupa.
Fig. 25.—The above in reduced scale.
Fig. 26.—Larva from a Musca domestica fly.

Plate V.—H. microstoma.

Fig. 27.—Larva from a Stomoxys calcitrans pupa.
Fig. 28.—The above (posterior end).
Figs. 29 and 30.—Larvae from a Stomoxys calcitrans pupa.
Fig. 31.—Larva from a Stomoxys calcitrans fly.
Fig. 32.—Larva from Stomoxys calcitrans fly.
Fig. 33.—The above (posterior end).

Plate VI.

Figs. 34 and 35.—Proboscis of Stomoxys calcitrans fly showing larval Habronema microstoma.

All figures, excepting 34 and 35, outlined with camera lucida.

Figs. 34 and 35, Photomicrographs of specimens fixed in alcohol and mounted in glycerine jelly.

Fig. 18, from specimens fixed in lacto-phenol.

Figs. 19-30, inclusive, from life.

Figs. 31-33, inclusive, from specimens fixed in alcohol and mounted in glycerine jelly.

Plate VII.—H. megastoma.

Fig. 36.—Egg and embryos from female.
Fig. 37.—Embryos from stomach tumour.
Fig. 38. Embryos from a tumour after fifteen days' incubation in faeces.
Fig. 39.—Larva from a Musca domestica larva.
Figs. 40 to 45.—Larvae from Musca domestica flies.

Plate VIII.

Figs. 46, 47. Larvae from Musca domestica flies.
Fig. 48.—Larva from a Musca domestica fly.
Fig. 48a.—The above (posterior end).
Fig. 49.—Larva from a stomach tumour.
Fig. 49a.—The above (posterior end).

All figures outlined with camera lucida.

Figs. 36-39 and 41-43 inclusive from life.

Figs. 40 and 44-49a inclusive from specimens fixed in alcohol and mounted in glycerine jelly.

TABLE I.

HABRONEMA MUSCAE FROM MUSCA DOMESTICA FLIES (ADULTS).

	1	2	3	4	5	6	7	8	9	10	11	12
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
Length	2.541	2.475	2.376	2.376	2.310	2.310	2.079	2.013	1.848	1.485	1.435	.800
Diameter, about .040 mm. from anterior end	.049	.040	.039	.039	.029	.033	.033	.033	?	?	.029	.033
Diameter, at anal opening	.036	.033	?.026	?	.029	.026	?	?	?	.030	?.026	?
Diameter, maximum (at end of oesophagus)	.056	.066	.050	.049	?	.052	.066	.056	.050	?	.043	?
Oesophagus, base from oral opening	.970	.910	.900	.920	.830	.860	.740	.775	.640	.460	.430	.300
Oesophagus, least diameter (at anterior end)	.010	.013	.013	?	.016	.016	.016	?.010	.013	.013	.013	?
Oesphagus, greatest diameter (at base)	.049	.049	.036	.039	.033	.033	.036	.030	.023	?	.020	?
Anal opening from tip of tail	.082	?	.089	?	.089	.089	.089	?	.079	.053	.050	?
Nerve ring from anterior end	?.135	.140	.132	.132	?.135	.138	.122	.122	.120	.092	.102	.100
Pharynx, length of	.046	.046	.046	.049	.046	.049	.043	.043	.043	.030	.030	.020
Tip of tail	spinous	spinous	spinous	spinous	spinous	spinous	spines under cuticle	spines under cuticle	not spined	not spined	not spined	not spined
Anal operculum	absent	absent	absent	absent	absent	absent	present	present	present	present	present	present

The above larvae were fixed in 70% alcohol and mounted in glycerine jelly before measurements were taken. Specimens 7, 8, 9 and 10 were in a moulting condition.

TABLE II.

HABRONEMA MICROSTOMA FROM STOMOXYS CALCITRANS (ADULTS AND PUPAE).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
Length	1.815	1.800	1.740	1.716	1.716	1.600	1.586	1.584	1.584	1.584	1.584	1.570	1.485	0.950
Diameter, about .040mm. from anterior end	?	.033	?	?	.030	.030	.030	.030	?	?	?	.030	?	.020
Diameter, at anal opening	?	?	?	?	.039	.030	.036	.030	.030	.030	.030	?	.033	.030
Diameter, maximum (at end of oesophagus)	.046	.072	.066	?	.063	.049	.046	.053	.049	.043	.049	.049	.049	.039
Oesophagus, base from oral opening	.750	.770	.740	.792	.740	.650	.680	?.680	.670	.680	.670	.640	?.700	.380
Oesophagus, least diameter (at anterior end)	.013	?	.013	.013	.013	.010	.020	.010	.010	.013	.013	?	.013	.010
Oesophagus, greatest diameter (at base)	.026	?	?	.036	.029	.026	.026	.033	.026	.030	.030	.030	.030	.016
Anal opening from tip of tail	?	.086	.066	?.082	.076	.066	.069	.066	.075	.056	.066	?	.059	.043
Nerve ring from anterior end	.120	?	?.132	.130	.110	.105	.102	?	.100	.102	.105	.108	.100	.092
Pharynx, length of	.053	.053	.049	.049	.053	.046	.043	.046	.046	.043	.046	.046	.049	.030
Tip of tail	spinous	spinous	spinous	spinous	spinous	spinous	? spines under cuticle	spinous	spinous	spinous	spinous	spinous	spines under cuticle	not spined
Anal operculum	absent	absent	absent	absent	absent	absent	present	absent	absent	absent	absent	absent	present	present

The above larvae were fixed in 70% alcohol and mounted in glycerine jelly before measurements were taken.
Specimens 7, 13 and 14 were in a moulting condition.
Specimens 7 and 13 were found in well-developed pupae: the remainder were from adults.
Six other larvae (from pupae) measuring from 1.419 mm. to 1.551 mm. were in similar condition to specimens 7 and 13.

TABLE III.

HABRONEMA MEGASTOMA FROM MUSCA DOMESTICA FLIES (ADULTS).

	1	2	3	4	5	6	7	8	9	10
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
Length	2.244	1.848	1.650	1.280	1.221	1.188	1.080	0.990	0.980	0.870
Diameter, about .040 mm. from anterior end	.049	.033	.035	.030	.029	.026	.029	.026	?	.029
Diameter, at anal opening	?.049	.039	.039	.036	.036	.039	.036	?	?	?
Diameter, maximum (at end of oesophagus)	.096	.082	.053	.043	.049	.046	.043	.046	?.053	.046
Oesophagus, base from oral opening	.630	.560	?.428	.330	?.340	.330	.290	.260	? 303	?.247
Oesophagus, least diameter (at anterior end)	.020	.016	.020	.013	?.010	.016	.013	.013	.010	.013
Oesophagus, greatest diameter (at base)	.049	.036	.023	.020	?	.023	.020	?	.016	.020
Anal opening from tip of tail	?	?.073	.069	.056	?	.063	.063	.066	?	?
Nerve ring from anterior end	.138	.132	.120	.099	.099	.109	.099	.099	.099	.073
Pharynx, length of	.066	.040	.033	.036	.030	.033	.030	.026	.026	.016
Tip of tail	spinous	spines under cuticle	not spined	not spined	not spined	not spined	not spined	not spined	not spined	not spined
Anal operculum	absent	present	present	present	present	present	present	present	present	present

The above larvae were fixed in 70% alcohol and mounted in glycerine jelly before measurements were taken.
Specimens 2 and 8 were in a moulting condition.
Specimen 2 was found in a cyst.

TABLE IV.

LARVAL HABRONEMA MEGASTOMA FROM STOMACH TUMOURS.

	1	2	3	4	5	6
	mm.	mm.	mm.	mm.	mm.	mm.
Length	2.805	2.805	2.772	2.739	2.640	2.310
Diameter, at about .040 mm. from anterior end	.049	.052	.049	.013	.049	.049
Diameter, at anal opening	.049	.056	.046	?	.049	?
Diameter, maximum (at end of oesophagus)	.115	.099	.118	.089	.099	.122
Oesophagus, base from oral opening	.750	.700	.750	.680	.650	.580
Oesophagus, least diameter (at anterior end)	.022	.016	.020	.019	.022	.022
Oesophagus, greatest diameter (at base)	.049	.059	.050	.046	.056	.059
Anal opening from tip of tail	.105	?.118	.105	?	.118	?
Nerve ring from anterior end	.181	.165	.165	.165	.165	.148
Pharynx, length of	.082	.082	.082	.085	.089	.079
Tip of tail	spinous	spinous	spinous	spinous	spinous	spinous
Anal operculum	absent	absent	absent	absent	absent	absent

The above specimens were fixed in 70% alcohol and mounted in glycerine jelly before measurements were taken.

TABLE V.

HABRONEMA SP. FROM MUSCA DOMESTICA FLIES (ADULTS) EXPOSED TO MIXED INFECTION OF H. MUSCAE AND H. MICROSTOMA.

	1	2	3	4	5	6
	mm.	mm.	mm.	mm.	mm.	mm.
Length	2.145	2.178	1.586	1.500	1.386	1.353
Diameter, about .040 mm. from anterior end	—	—	—	—	—	—
Diameter, at anal opening	.033	?	.033	.043	.033	?
Diameter, maximum (at end of oesophagus)	.063	.053	.053	.069	.049	.059
Oesophagus, base from oral opening	.820	.800	.560	.550	.540	.500
Oesophagus, least diameter (at anterior end)	.013	?	.016	.016	.013	?
Oesophagus, greatest diameter (at base)	.033	.026	.030	.026	.026	.030
Anal opening from tip of tail	.099	?	.073	.063	?	?
Nerve ring from anterior end	.148	.132	.112	.108	.099	.102
Pharynx, length of	.049	.049	.020	.031	.026	.026
Tip of tail	spinous	spinous	?	?	not spined	not spined
Anal operculum	absent	absent	present	present	present	present

Specimens 1 and 2 probably H. muscae.
Specimens 3, 4, 5 and 6 probably H. microstoma.
The above larvae were fixed in 70% alcohol and mounted in glycerine jelly before measurements were taken.
Specimens 3-6 inclusive were in a moulting condition.

TABLE VI.

HABRONEMA MICROSTOMA (?) FROM STOMOXYS CALCITRANS (ADULTS) FROM MIXED INFECTION OF H. MUSCAE AND H. MICROSTOMA.

	1	2	3	4	5	6	7	8	9	10	11
	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.	mm.
Length	1.815	1.650	1.650	1.620	1.430	1.320	1.221	1.188	1.150	1.130	1.100
Diameter, at anal opening	.030	.033	.030	.033	?	?.043	.033	.036	.036	.033	.033
Diameter, maximum (at end of oesophagus)	.043	.0 6	.039	.043	.043	.043	?	.046	.049	.043	.050
Oesophagus, base from oral opening	.690	.730	?	.720	.660	.600	.530	.485	.430	.400	.370
Oesophagus, least diameter	.010	?	.013	?	.013	.013	.013	.013	.013	.013	.013
Oesophagus, greatest diameter	.023	.026	.023	.023	.023	.023	.026	.023	.026	.026	.026
Anal opening from tip of tail	.076	.073	.069	.069	?	?	.053	.053	.053	.046	.049
Nerve ring from anterior end	.109	.120	.115	.112	.115	.115	.105	.112	.112	.112	.109
Pharynx, length of	.049	.049	.049	.046	.046	.049	.046	.046	.030	.026	.023
Tip of tail	spinous	?	spinous	spinous	not spined	not spined	not spined	not spined	not spined	not spined	not spined
Anal operculum	absent	absent	absent	absent	?	?	present	present	present	present	present

The above larvae were fixed in 70% alcohol and mounted in glycerine jelly before measurements were taken. Specimens 2, 3, 5, 6, 7, 8, 9, 10 and 11 were in a moulting condition.

TABLE VII.

HABRONEMA MUSCAE (?) FROM MUSCA DOMESTICA FLIES (ADULTS) EXPOSED TO A MIXED INFECTION OF H. MUSCAE AND H. MICROSTOMA. (Cf. Table VIII.)

	1	2	3	4
	mm.	mm.	mm.	mm.
Length	2.277	2.277	2.244	2.178
Diameter, at anal opening	—	—	—	—
Diameter, maximum (at end of oesophagus)	.049	.049	.053	.049
Oesophagus, base from oral opening	.800	.770	.770	.800
Oesophagus, least diameter	.013	.015	—	.013
Oesophagus, greatest diameter	.030	.030	.031	0 28
Anal opening from tip of tail	—	.086	?.082	?.082
Nerve ring from anterior end	.115	.115	.122	.119
Pharynx, length of	.043	.043	.040	.043
Tip of tail	spinous	spinous	spinous	spinous
Anal operculum	absent	absent	absent	absent

The above larvae were fixed in 70% alcohol and mounted in glycerine jelly before measurements were taken.

TABLE VIII.

HABRONEMA MICROSTOMA (?) FROM STOMOXYS CALCITRANS FLIES (ADULTS) EXPOSED TO A MIXED INFECTION OF H. MUSCAE AND H. MICROSTOMA. (Cf. Table VII.)

	1	2	3	4	5	6
	mm.	mm.	mm.	mm.	mm.	mm.
Length	1.716	1.683	1.650	1.617	?1.584	1.584
Diameter, at anal opening	.030	.033	.030	.033	.030	.030
Diameter, maximum (at end of oesophagus)	.049	.049	.049	.049	.049	.053
Oesophagus, base from oral opening	.690	.710	.700	.660	.720	.650
Oesophagus, least diameter	.013	.010	.013	.013	.010	.010
Oesophagus, greatest diameter	.030	.026	.023	.026	.020	.027
Anal opening from tip of tail	.076	.076	.073	.076	.079	.073
Nerve ring from anterior end	.112	.105	.109	.099	.105	.102
Pharynx, length of	.046	.049	.049	.046	.049	.046
Tip of tail	spinous	spinous	spinous	spinous	spinous	spinous
Anal operculum	absent	absent	absent	absent	absent	absent

The above larvae were fixed in 70% alcohol and mounted in glycerine jelly before measurements were taken.

TABLE IX.

CENSUS OF NEMATODE PARASITES FOUND IN 39 HORSES' STOMACHS.

Horse.	Date.	H. muscae.	H. microstoma.	H. megastoma.
1	22/5/17	Absent	Absent	Present
2	31/5/17	P.	A.	P.
3	3/7/17	A.	A.	P.
4	9/7/17	P.	A.	P.
5	18/7/17	P.	A.	P.
6	10/8/17	A.	A.	P.
7	13/8/17	P.	A.	P.
8	16/8/17	P.	A.	A.
9	24/8/17	P.	A.	A.
10	29/8/17	P.	A.	A.
11	29/8/17	A.	A.	P.
12	3/9/17	P.	P.	P.
13	5/9/17	A.	A.	A.
14	6/9/17	P.	P.	A.
15	10/9/17	P.	A.	A.
16	1/10/17	P.	A.	A.
17	3/10/17	P.	A.	P.
18	8/10/17	P.	P.	P.
19	10/10/17	P.	P.	A.
20	22/10/17	P.	P.	A.
21	25/10/17	P.	P.	P.
22	29/10/17	P.	A.	A.
23	21/11/17	P.	A.	A.
24	26/11/17	P.	P.	P.
25	28/11/17	P.	P.	P.
26	3/12/17	P.	P.	P.
27	7/12/17	P.	A.	A.
28	13/12/17	P.	P.	P.
29	18/12/17	P.	A.	P.
30	21/12/17	P.	P.	A.
31	9/1/18	P.	A.	A.
32	11/1/18	P.	A.	A.
33	14/1/18	P.	P.	A.
34	18/1/18	P.	P.	P.
35	21/1/18	P.	P.	A.
36	23/1/18	P.	A.	P.
37	1/3/18	A.	A.	A.
38	5/3/18	P.	A.	A.
39	7/3/18	P.	A.	A.

PART II.

Certain points in the Life-History of Melophagus ovinus, Linn., the Sheep Louse-fly, or "Sheep-Tick."

Introduction.

The life-history and habits of the Sheep Louse-fly, or "Sheep-Tick" (Melophagus ovinus Linn.) have been known for many years as a result of investigations carried out in Europe and U.S.A. Prior to 1916 there do not appear to have been any similar investigations carried out here, and as there was no known marked difference in Australia from the life-history of the pest as known elsewhere, legislation for the control of the Sheep Louse-fly by dipping was introduced accordingly. The enforcement of the Sheep Dipping Act, however, has not had the effect of eradicating the pest. Many sheep-owners contend that this failure is due to the fact that the Louse-flies and their pupae become dislodged from the fleece of the host and remain viable in the grass, brushwood, or elsewhere, for a longer or shorter period, during which the pupae develop into young Louse-flies, which, with similarly dislodged adults, subsequently infect "Tick"-free sheep.

In view of the importance of this contention it seemed desirable that the points in question as well as others in the life-history and habits of this pest should be investigated. With this object in view, certain experiments were begun by Sweet and Seddon in this Institute in the summer of 1916, but though they showed some difference from observations elsewhere they were still incomplete, and required confirmation and extension. The present writer therefore commenced a series of observations and experiments in May, 1917, to verify under a variety of conditions, the work of Swingle in U.S.A. and Sweet and Seddon in Victoria, and also to determine the period of viability of the pupa when removed from the host. The chief subjects upon which further information was sought were :—

(1) Incubation period of the pupa on the host.

(2) The period required by the young Louse-fly to reach maturity —i.e, the period elapsing between its emergence from the parent and the extrusion of the first offspring.

(3) The length of life of the female fly.

(4) The number of pupae extruded by an individual female and the time elapsing between each extrusion.

(5) The period of viability of the insect when removed from the host, (a) when under one day old and unfed, (b) when between three and seven days old, and (c) in the adult condition.

(6) The period of viability of the pupa when removed from the host and kept under varying conditions.

Historical.

The sheep Louse-fly is one of the best known external parasites of the sheep, and has long been known in Europe and America as a pest of some considerable importance. The life history and habits of the fly are referred to briefly and more or less vaguely in many text-books and other publications. In many of these the writers express widely different views upon such important details as the length of life of the female Louse-fly, the number of pupae extruded by the female and the intervals between each extrusion. Dr. Cooper Curtice (1890), however, gives a more detailed account of the habits of the parasite, and some of his observations will be referred to later on in this Report.

The records of investigations and observations by Dr. L. D. Swingle (1913) are the most complete so far published. This investigator, after some initial failures, was able to obtain precise information on many points in the life-history of the parasite, which appear to have been merely conjectured hitherto. He showed that the period which elapsed between the deposition of the pupa, or more correctly the larva, to the emergence of the young Louse-fly was 19-23 days in summer, and 19 to 36 days in winter. Young males and females, he found, are capable of copulating within three or four days after their emergence, and the female reaches maturity, i.e., extrudes the first pupa, fourteen to thirty days after its emergence from the puparium. There is great mortality among the young "ticks" before they take their first meal, but he was able to keep female "ticks" under observation for five and a-half months, and considered that some of them probably live much longer, although many die earlier.

The rate of pupa laying, counting from the deposition of the first one, was found to be one pupa every seven or eight days. The number of pupae laid by an individual female depends upon the length of her life. For a female living four months, which he considered might be regarded as an average life-time, the number is about ten to twelve pupae. For one living six months the number is fifteen or more.

Professor W. B. Herms refers briefly to the Louse-fly, remarking that when off the sheep the insects die in from two to eight days.

The investigations made by Dr. Georgina Sweet and Mr. H. R. Sedden, B.V.Sc. (1917) dealt with one aspect of the subject, namely, the viability of the sheep Louse-fly when removed from the host. These authors refer to the statements of earlier investigators and authors as showing that the life of the parasite off the host and deprived of its food is generally believed to be from two to seven days. They were unable to find any record of sheep Louse-flies being kept alive under these conditions to the eighth day. Their own investigations, which are recorded in detail, showed that under certain conditions the Louse-fly lived for varying periods up to eleven and three-quarter days off the host and without food. The condition found to be most favourable was the moderately cool, uniform temperature of a cellar. Two groups of insects were kept under such conditions, one group on bare soil and the other on soil on which rested a few dry leaves. The first group were all dead in eleven days, whilst the second group were all dead in eleven and three-quarter days. At the same time another group was kept on wool in the cellar; these were all dead in four days. In one experiment in early December (summer), a group of Louse-flies was placed on a moist sod of lawn grass out of doors; these were all dead in five and three-quarter days. In two other experiments out of doors, all the insects died in two and three-quarter days.

1. Experiments to determine the incubation period of the pupae.

Experiment No. 1.

Eleven pupae extruded during the preceding twelve hours were placed (May 30th) in a small muslin bag secured to the fleece on a sheep's back, $\frac{1}{4}$ in. to $\frac{1}{2}$ in. from the skin and $2\frac{3}{4}$ in. to 3 in. from the surface of the wool, the sheep being housed in a brick stall, in which the temperature ranged during the experiment from 45°F. to 47°F. Two young Louse-flies emerged on the twenty-second day, three on the twenty-third day, and three on the 24th day. Three pupae failed to produce young.

Experiment No. 2.

Twelve pupae extruded between 2 p.m. on September 10th and 5 p.m. on September 11th (early spring), by females enclosed within a small area of wool on a sheep's back, produced four young flies on

the nineteenth day, five on the twentieth day, and two on the twenty-first day. The remaining pupa was infertile. The area of wool, 2 in. in diameter, was enclosed in a metal ring 3 in. in diameter and 1 in. deep, secured to the skin with pitch plaster. The wool was $\frac{3}{4}$ in. long and the pupae were laid by the females about the middle of the staple. The stall used was the one referred to above, and in it the temperature ranged from 47°F. to 72°F. during the experiment.

Summary and Observations.

The incubation period of the pupa on the host has been found to be twenty-two to twenty-four days during the winter months, May and June, when the temperature varied between 43°F. and 47°F. and nineteen to twenty-one days during the spring months, September and October, when the temperature ranged from 47°F. to 72°F.

Swingle found in U.S.A. that the incubation period of the pupa on a sheep kept in a barn was twenty to thirty-six days in winter, when the average minimum temperature was 7.2°F. and the average maximum temperature 27.3°F. In summer, when the average minimum temperature was 44°F. and the average maximum temperature 74°F., the period was nineteen to twenty-three days. He considered that were the sheep turned out of doors in winter, the period might be increased to forty or forty-five days in some cases.

2. To determine the period required by the young Louse-fly to reach maturity, i.e., the period elapsing between its emergence and the extrusion of the first pupa.

Experiment No. 3.

On May 21st one male and eight female "ticks" which emerged from their pupae on the previous day were placed in a small area of wool on a sheep's back. The temperature in the sheep-pen, which was similar to the one referred to on page 79, ranged from 44°F. to 66°F. during the experiment. The wool was about $\frac{3}{4}$ in. long and the enclosed space about $2\frac{1}{2}$ in. in diameter. The surrounding wool was closely shorn to allow a clear space of six inches around the $2\frac{1}{2}$ in. circle of standing wool. Vaseline was then smeared freely on the shorn portion with the object of preventing the young "ticks" escaping. On the following day (May 22nd), several adult "ticks" from other parts of the host were found in the area under-

observation. These were destroyed, and six young male "ticks" of unknown ages were liberated with the nine young "ticks" in the enclosure. To prevent the escape of the fifteen "ticks," a piece of muslin was placed over them and the edges fastened down to the skin with liquid glue. The glue did not hold very securely, and it was found necessary to renew the muslin daily. On May 26th three of the young "ticks"—the only surviving females—were mating. On May 30th young larvae were evidently present in the abdomen of all three. On June 4th three pupae were found attached to the wool about $\frac{1}{2}$ in. from the skin. One of these pupae had been extruded very recently, as shown by its pale colour. The muslin cover was then removed, and a collar or cup of buckram $1\frac{1}{2}$ in. deep by $2\frac{3}{4}$ in. in diameter, and covered with a muslin top was substituted. The enclosure was accidently stripped off during the following night, allowing the "ticks" to escape. Thus three "ticks" which emerged on May 21st (early winter) mated five days later, and extruded their first pupa when thirteen days old.

Experiment No. 4.

On January 12th (mid-summer) sixteen young "ticks," about twelve hours old, were liberated on a lamb housed in one of the pens previously referred to. The lamb had been dipped on December 17th, and shorn and re-dipped on January 3rd. When the experiment commenced the fleece was $\frac{1}{2}$ in. long and free of both living and dead "ticks." The first examination was made three days later (January 15th), when several dead "ticks" were found. The living "ticks" were not counted daily on account of the difficulty experienced in finding them. On the eleventh day (January 23rd) two pairs were found copulating. On the twenty-first day (February 2nd) two pupae were found in the fleece. On the twenty-second day (February 3rd) another pupa was found. On the thirty-first day (February 12th) two more pupae were found. Another was found on the thirty-second day (February 13th). On the thirty-ninth day (February 20th) the host was carefully examined with the object of finding and removing all the surviving "ticks" and their pupae. These numbered three females, six males and two pupae. One of the females contained a large larva, apparently nearly ready for extrusion. These nine "ticks" were then placed on another lamb, previously freed of "ticks," for further observation (see p. 84). Great difficulty was experienced in finding the "ticks" and their pupae, even in such short wool. In this experiment it would appear

that the young "ticks" first mated on the eleventh day, extruded the first pupae about the twenty-first day, their second pupae about the thirty-first day, and their third pupae about the thirty-ninth day.

Experiment No. 5.

An experiment similar to the above in all particulars excepting in the number of "ticks" used was commenced on January 23rd, with twenty-five "ticks," about twelve hours old. The fleece was searched daily, and many dead "ticks" were removed, especially during the first few days. On the fourteenth day (February 6th) one pair was found copulating. On the twentieth day (February 12th) the fleece was carefully searched for "ticks" and their pupae, with the result that only five "ticks" could be found. On the twenty-third day (February 15th) two pupae were found. On the thirty-second day (February 24th) the fleece was very carefully searched to ascertain the number of surviving "ticks," which was three males and two females. Two pupae were found also.

Thus assuming that copulation did not take place earlier than February 6th, and that the four pupae were all extruded by the two females found on February 24th, it will be seen that the first mated when about fourteen days old, extruded the first pupae about the twenty-third day, and the second pupa about the thirty-second day.

Many other experiments similar to, or modifications of, Experiment No. 3, page 80, were undertaken, but in none of them was I able to obtain any useful data. It was found impossible to firmly secure a buckram or muslin enclosure to the host for more than a day at the longest. In one experiment only (No. 3, p. 80) could the "ticks" be kept alive and under observation for a sufficiently long period to enable me to obtain the desired information, and after repeated failures this method was abandoned. Collars made of tin or modelling wax were then tried. With the aid of melted pitch plaster it was found possible to secure these enclosures in position for several days, and, further, as the "ticks" were unable to crawl up the smooth inner surface, it was not necessary to cover the top of the enclosure. The young "ticks," however, soon died in these enclosures, and in no case did one live to reach maturity. The writer's experience with "ticks" confined in small enclosures on the host agree with Swingle's, i.e., such "ticks" will not live for more than a couple of weeks, very rarely so long.

Summary and Observations.

It will be seen from these experiments that the young Louse-fly or "tick" may reach maturity during the early winter thirteen days after its emergence from the pupa. In one experiment (No. 3, p. 80) the young "ticks" were kept very close together, and therefore had an opportunity of copulating as soon as they were sufficiently developed. Copulation took place on the fifth day after their emergence.

In two other experiments (Nos. 4 and 5) carried out in midsummer the young "ticks" copulated on the eleventh and fourteenth days respectively, and reached maturity, i.e., extruded the first pupa, on the twenty-first and twenty-third days respectively. In these two experiments the few "ticks" under observation had access to all parts of the host's fleece, and appeared to be constantly moving from one part of the body to another. Under these conditions the chance of early mating would be greatly reduced, and it is believed that this fact alone accounted for the long period elapsing between their emergence and the deposition of the first pupa as compared with the period ascertained in Experiment No. 3, in which the young "ticks" were kept close together. Swingle (1913, p. 16) found that young "ticks" marked with a silk thread tied around the constriction between the body and thorax and then liberated on the host copulated on the fourth or fifth day during the winter, when the average maximum temperature was 27.3° and the average minimum temperature was 7.2.°. He observes also (p. 23) that young male and female "ticks" are capable of copulating when three days old.

The same investigator (page 18) found that the young female "tick" required a minimum period of fourteen days in which to reach maturity, but he considered that the period might possibly be reduced by carrying out experiments with thousands of "ticks."

3. To determine the length of life of the female.

As previously stated, it was found impossible to keep the "ticks" for any lengthy period within a confined area upon the host; those that were unable to escape soon perished. In one experiment (No. 3, p. 80) young "ticks" were kept for two weeks in an enclosed area, but in all others they died or escaped within a week or less. It was apparent therefore, that some method would have to be devised which would allow the "ticks" a much greater measure of liberty if the desired information was to be obtained.

Following Swingle, the present writer marked some young "ticks" by sticking a very small piece of coloured paper on the dorsum of the abdomen, others with coloured adhesive substances and stains. None of these methods, however, was sufficiently permanent to be of any service. The following method employed successfully by Swingle was then tried :—A short piece of silk thread was separated into its three component strands and tied around the young "ticks" between the thorax and abdomen. The ends were then snipped off close to the knot and the "ticks" were liberated on a lamb previously freed of "ticks." For this purpose young "ticks" which had been fed were employed, as they were less liable to be damaged than very young ones. In most cases the marked "ticks" found their way to the surface of the wool and remained there until they died. In others they remained in the fleece, but did not thrive, and ultimately died. Swingle remarks (p. 21), ". . . if a dozen female 'ticks' be placed in the wool within a circle having a diameter of two inches, the following day they will most generally be found very near that area, at least within a radius of three or four inches." This has not been the present writer's experience here. Even during the winter months the young "ticks" especially showed a very noticeable inclination to wander freely and rapidly, even when encumbered as described above. This fact accounts in a large measure, if not entirely, for the writer's want of success in these experiments.

As a last resort a lamb was shorn and freed of "ticks" after which sixteen young "ticks" were liberated in the fleece (January 12th). The history of these "ticks" up to January 20th is recorded in Experiment No. 4, page 81, where it will be seen that only nine of the original sixteen "ticks" could be found at the end of thirty-nine days. Of those found only three were females. It was intended to keep these nine "ticks" under observation with a view to ascertaining how long they would live and how many pupae each would extrude during its life. As stated on page 81, two of the three "ticks" had already extruded three pupae each, the third contained a larva nearly ready for extrusion. On February 20th these three "ticks" were liberated on a lamb previously shorn and freed of these parasites. On the following day the "tick" containing the large larva was found dead. On the forty-eighth day (March 1st) one pupa was found, but one of the two remaining females could not be accounted for. After two more unsuccessful attempts to find the missing "tick" the experiment was abandoned.

The history of another experiment similar to the above is recorded on p. 82, Experiment No. 5. In this experiment only five "ticks" could be traced at the end of the thirty-second day (February 24th), during which period the two surviving females each extruded two pupae. On February 24th these five "ticks" were liberated on a lamb previously freed of "ticks." On February 26th and two subsequent days only one female and two or three males could be accounted for.

Three other similar experiments were commenced in February and March, but as satisfactory results could not be obtained they were abandoned a few weeks later.

4. To determine the number of pupae extruded by an individual female and the time elapsing between each extrusion.

Attempts to determine the length of life of the female Louse-fly having failed, it is obvious that the number of pupae extruded by an individual female could not be determined. The results of two experiments, however, may be referred to again as indicating the periods elapsing between extrusion of the first and subsequent pupae.

In Experiment No. 4 three young "ticks" which were liberated on a lamb in mid-summer (January 12th) each extruded the first pupa about the twenty-first day, the second pupa about the thirty-first day, and the third pupa (in the case of two "ticks") about the thirty-ninth day. In Experiment No. 5, two young "ticks" each extruded the first pupa about the twenty-third day, and the second pupa about the thirty-second day.

Thus the period which elapsed between the extrusion of each pupa was about nine days (9.14 days). These periods are reckoned from the date of finding of the pupa, which in some cases may have been a day or more after their extrusion; they should be regarded therefore as approximate only. Swingle's investigations in America (1913, pp. 19 and 20), showed that the average rate of pupa laying was one about every eight days (7.89 days in one experiment and 7.99 days in another).

5. To determine the period of viability of the Sheep Louse-fly when removed from the host, (a) unfed flies under one day old, (b) flies from three to seven days old which had fed, (c) adult flies.

A series of experiments with forty-six groups of Sheep Louse-flies kept under thirteen different sets of conditions was commenced in

May, 1917, and continued during the following June, November and December. The insects in Group A were reared from pupae in the laboratory, whilst those in Groups B and C were collected on the host. The ages of the "ticks" in Group B were judged only from their appearance and size. In Group C the required number of adults was selected from mated pairs.

Nature of Receptacles and the conditions under which the "Ticks" were kept.

Experiment No. 6.

A sod of lawn grass in a Petri dish, with a glass chimney 6.4 cms. high and 6.2 cms. wide, firmly embedded in the middle to form an enclosure. The enclosed area was kept moist by water poured on the sod outside the chimney. This receptacle was kept in a cellar under the Institute buildings.

Experiment No. 7.

This receptacle was prepared in the same manner as No. 6, but it was placed under a table on the lawn within the Institute quadrangle.

Experiment No. 8.

Soil in Petri dish, pressed down to form a level surface, upon which a few dry leaves were placed. Position as in No. 7.

Experiment No. 9.

Receptable prepared as in No. 8, but placed in cellar, as in No. 6.

Experiment No. 10.

Prepared similarly to Nos. 8 and 9, excepting that no leaves were placed on the surface of the soil. The position was on the lawn, as in Nos. 7 and 8.

Experiment No. 11.

As in No. 10, but placed in cellar, as in Nos. 6 and 9.

Experiment No. 12.

Sheep's wool (about one handful) placed in an open Petri dish on laboratory table.

Experiment No. 13.

Sheep's wool, as above, but kept in cellar.

Experiment No. 14.

A small wooden box measuring $6\frac{1}{4}$x4x$3\frac{1}{2}$ in., containing one inch of sheep-yard sweepings resting upon one inch of moist soil, the top covered with muslin, placed on the wooden floor of a shelter-house in the sheep yard.

Experiment No. 15.

A wire gauze cage 4 in. in diameter by 5 in. high resting on the natural surface of a sheep-yard, which was sheltered on one side by a wall but otherwise exposed to all weather conditions excepting the rays of the sun during the afternoon.

Experiment No. 16.

A piece of dry Eucalyptus tree trunk, 9 in. long by $2\frac{1}{2}$ in. in diameter, covered with loose bark divided lengthwise into two segments and held in position by rubber bands, the whole standing vertically upon a layer of moist sheep-pen sweepings in a dish and placed in the cellar.

Experiment No. 17.

As in No. 16, but placed upon a table on the lawn, as in Nos. 7, 8, and 10.

Experiment No. 18.

A square jar about 7 in. across the opening, containing about 2 in. of moist sheep-pen sweepings, and placed on the floor of a shelter-house in sheep yard, as in No. 14.

Details of these experiments are recorded in the following statement :—

No.	Position	No. and Age.	Number and sex of "ticks" which died during each following 24 hours, or fraction of 24 hours. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
6A	Lawn Grass. In cellar	10 under 1 day old	* 50° $3^{2\,♂}_{1\,♀}$	50° $1\,♂$	... ...	51.5° $2\,♂$	53° ...	53° $1\,♂$	51° ...	51° ...	50° $2\,♂$	... ...	51° $1\,♀$	... ...	... ...	... ...	... ...
6B	Lawn Grass. In cellar	25 about 7 days old	* 50° $2\,♂$	50° $1\,♂$	... ...	51.5° $1\,♂$	53° $3^{2\,♂}_{1\,♀}$	53° $7^{6\,♂}_{1\,♀}$	51° $2\,♂$	51° $6^{3\,♂}_{3\,♀}$	50° $1\,♂$	.. ...	51° $1\,♀$	50° $1\,♀$	... ...	... ...	... ...
6C	Lawn Grass. In cellar	25 adults	* 50° ...	50° ...	... ...	51.5° $3\,♀$	53° $4^{1\,♂}_{3\,♀}$	53° $5^{4\,♂}_{1\,♀}$	51° $4^{2\,♂}_{2\,♀}$	51° $2\,♀$	50° $2\,♀$	... $1\,♀$	51° ...	50° ...	... ...	... ...	... ...
7A	Lawn Grass. On lawn	10 under 1 day old	‖ 42° $4^{3\,♂}_{1\,♀}$	39° $2\,♂$	... ...	42° $3^{2\,♂}_{1\,♀}$	56° ...	... $1\,♀$	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...
7B	Lawn Grass. On lawn	25 about 7 days old	‖ 42° $2\,♂$	39° $2\,♂$	... ...	42° $14^{10\,♂}_{4\,♀}$	56° $4^{3\,♂}_{1\,♀}$	... $2\,♂$	... $1\,♀$	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...
7C	Lawn Grass. On lawn	18 adults	‖ 42° ...	39° $1\,♂$	... ...	42° $3^{2\,♂}_{1\,♀}$	56° $4^{2\,♂}_{2\,♀}$	... $3^{2\,♂}_{1\,♀}$	... $1\,♀$	... $4^{1\,♂}_{3\,♀}$	... $2\,♀$	... ...	... ...	... ...	... ...	... ...	... ...
8A	Soil, with leaves on surface. On lawn	2 under 1 day old	‖ 32.5° ...	... ...	42° $1\,♂$	48° ...	40° ...	45° $1\,♀$	.. ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...
8B	Soil, with leaves on surface. On lawn	2 under 1 day old	‖ 51° ...	50° ...	42° $1\,♀$	35° $1\,♀$	... ..	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ..

Nos. 6A, 6B, 6C, 7A, 7B, 7C, late June; 8A, late May; 8B, early June.

* Minimum Temperature (Fahr.) in Cellar. ‖ Minimum Temperature (Fahr.) in Sheep Yard.

No.	Position	No. and Age.	Number and sex of "ticks" which died during each following 24 hours, or fraction of 24 hours. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
8C	Soil, with leaves on surface. On lawn	19 about 3 days old	‖ 32.5° ... 1♂ ...		42° 6^{3}_{3}	48° 1♂	40° 6^{3}_{3}	45° 1♂	51° 3♂	52° 1♀	... ...	... ...	... ...	... ...	... ...	... ...	... ...
8D	Soil, with leaves on surface. On lawn	20 adults	‖ 35°		46° 2♂ 1♀	44° 3^{1}_{2}♂ 2♀	44° 6^{3}_{3}♂ 3♀	48° 1♂	47° 1♀	... 4^{3}_{1}♂ 1♀	... ...	46° 3♀	... ...	... ...	... ...	... ...	... ...
9A	Soil, with leaves on surface. In cellar	8 under 1 day old	* 50° 51°		... ...	51° ...	52° ...	53° ...	53° ...	54° 1♂	56° 2♂ 2♀	... ...	1♀ ...	56° 2♀	56° 1♀	57° 1♀	... ...
9B	Soil, with leaves on surface. In cellar	6 about 3 days old	* 50° 51°		... ...	51° ...	52° 1♂	53° 2♂ 2♀	53° 1♂	54° 1♀	56° 1♀	... ...	... ...	... ...	... ...	... ...	... ...
9C	Soil, with leaves on surface. In cellar	26 adults	* 51°		51° 1♂	52° 8^{3}_{5}♂ 5♀	53° 4^{1}_{3}♂ 3♀	53° 4^{2}_{2}♂ 2♀	54° 1♀	56° 3♀	... ...	... 3^{1}_{2}♂ 2♀	56° 1♀	56° 1♀	... ...	... ...	... ...
10A	Soil, no leaves on surface. On lawn	5 under 1 day old	‖ 35°		46° 1♀	44° 2♂ 2♀	44° 2♂ 2♀	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...
10B	Soil, no leaves on surface. On lawn	9 about 5 days old	‖ 50° 42°		35° 4♂	... ...	46° 5^{3}_{2}♂ 2♀	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	.. ...	... ...
10C	Soil, no leaves on surface. On lawn	25 about 7 days old	‖ 35° 32.5° ... 1♂		43° 6♂	42° 13^{7}_{6}♂ 6♀	39° 2♂ 2♀	... ...	42° 3^{1}_{2}♂ 2♀	... ...	... ...	... ...	... ...	... ..	... ...	... ...	... ...

Nos. 8C, 9A, 9B, 9C, late May; 8D, 10A, 10B, early June; 10C, late June.

‖ Minimum Temperature (Fahr.) in Sheep Yard * Minimum Temperature (Fahr.) in Cellar.

No.	Position	No. and Age.	Number and sex of "ticks" which died during each following 24 hours, or fraction of 24 hours. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
10D	Soil, no leaves on surface. On lawn	16 adults	‖ 35°	...	46°	44°	44°	48°	47°	...	...	46°	...	...	...	...	...
			...	...	1♀	6♂ 4♀	4♂ 3♀	1♀	2♀	...	...	2♀	...	...	...	...	...
11A	Soil, on leaves on surface. In cellar	5 under 1 day old	* 54°	...	53°	53°	50°	50°	53°	...	..	...	...	...	...	...	...
			...	...	...	2♂ ♀	1♀	1♂	1♂	...	...	...	...	...	...	...	...
11B	Soil, on leaves on surface. In cellar	8 about 5 days old	* 57°	54°	54°	...	53°	53°	50°	50°	53	46°	...	...	...	...	...
			...	...	1♂	...	2♂ 2♀	...	1♂	1♂	...	...	...	1♀	...	...	...
11C	Soil, no leaves on surface. In cellar	20 adults	* 54°	...	53°	53°	50°	50°	53°	...	...	52°	52°	50°	...	...	...
			...	...	1♂	3♂ 1♀	2♀	4♂ 3♀	...	3♂ 2♀	...	4♂ 1♀	1♂	2♀	...	...	...
12A	Wool on Laboratory table	10 under 1 day old	‡ 52°	...	...	...	...	...	...	...	...	...	...	...	...	...	...
			10♂ 7♀	...	...	...	...	...	...	...	...	...	...	...	...	...	...
12B	Wool on Laboratory table	10 about 7 days old	‡ 45°	...	45°	50°	...	...	...	...	...	...	...	...	...	...	...
			3♂ 2♀	...	6♂ 4♀	1♂	...	...	...	...	...	...	...	...	...	...	...
12C	Wool on Laboratory table	102 adults	‡ 52°	48°	...	...	...	...	...	...	...	...	...	...	...	...	...
			94♂ 53♀	8♂ 3♀	...	...	...	...	...	...	...	...	...	...	...	...	...
12D	Wool on Laboratory table	25 adults	‡ 43°	42°	...	...	...	...	...	...	...	...	...	...	...	...	...
			18♂ 11♀	7♀	...	...	...	...	...	...	...	...	...	...	...	...	...

Nos. 10D, 11A, 11B, 11C, 12A, 12B. 12C, early June; 12D, late June.

* Minimum Temperature (Fahr.) in Cellar. ‖ Minimum Temperature (Fahr.) in Sheep Yard ‡ Minimum Temperature (Fahr.) in Sheep Stall.

No.	Position	No. and Age.	Number and sex of "ticks" which died during each following 24 hours, or fraction of 24 hours. 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
13A	Wool in cellar	7 under 1 day old	* 57°	54°	...	...	...	...	...	...	...	...	...	...	...	...	...
			$6^{3\,♂}_{3\,♀}$	$1_{♀}$	...	...	...	...	...	...	...	...	...	...	...	...	...
13B	Wool in cellar	10 about 7 days old	* 57°	...	...	...	...	...	...	...	...	...	...	...	...	...	...
			$10^{5\,♂}_{5\,♀}$	...	...	...	...	...	...	...	...	...	...	...	...	...	...
13C	Wool in cellar	20 adults	* 57°	54°	...	...	...	...	...	...	...	...	...	...	...	...	...
			$18^{4\,♂}_{14\,♀}$	$2_{♀}^{♂}$	...	..	...	...	...	...	...	...	...	...	...	...	...
13D	Wool in cellar	25 adults	* 49°	50°	...	...	...	...	...	...	...	...	...	...	...	...	...
			$8^{4\,♂}_{4\,♀}$	$17^{8\,♂}_{9\,♀}$	...	...	...	...	...	...	...	...	...	...	...	...	...
14A	Sheep-pen sweepings in sheep shelter	12 under 1 day old	‖ 52°	51°	...	50°	42°	35°	...	46°	...	...	...	...	...	...	...
			$1_{♂}$	$2_{♂}$	$2_{♀}^{♂}$	$1_{♂}$	$3^{1\,♂}_{2\,♀}$	...	$3^{1\,♂}_{2\,♀}$	...	...	...	...	...	...	...	...
14B	Sheep-pen sweepings in sheep shelter	7 about 7 days old	‖ 50°	42°	35°	...	46°	46°	44°	...	...	...	...	...	...	...	...
			...	$1_{♂}$	$2_{♀}^{♂}$	...	$1_{♂}$	$2_{♀}^{♂}$	$1_{♂}$	...	...	...	...	...	...	...	...
14C	Sheep-pen sweepings in sheep shelter	20 about 7 days old	‖ 35°	32.5°	43°	42°	39°	...	42°	56°	...	...	...	...	...	...	...
			...	$11^{5\,♂}_{6\,♀}$	...	$3^{2\,♂}_{1\,♀}$	$1_{♂}$	...	$3^{2\,♂}_{1\,♀}$	$1_{♂}$	$1_{♀}$	...	...	...	...	...	...
14D	Sheep-pen sweepings in sheep shelter	11 adults	‖ 50°	42°	35°	...	46°	44°	44°	48°	47°	...	...	...	...	...	...
			...	...	$2_{♀}$	...	$7_{♀}$	..	...	$1_{♀}$	...	$1_{♀}$	...	...	...	...	...

Nos. 13A, 13B, 13C, 14A, 14B, 14D, early June; 13D, 14C, late June.

* Minimum Temperature (Fahr.) in Cellar. ‖ Minimum Temperature (Fahr.) in Sheep Yard.

No.	Position	No. and Age.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
			Number and sex of "ticks" which died during each following 24 hours, or fraction of 24 hours.														
15A -	Surface soil in sheep -	10 under -	‖ 50°	42°	35°	...	46°	44°	44°	...	...	...	...	...	...	...	...
	yard	1 day old	...	1 ♂	3 1♂ 2♀	...	3 2♂ 1♀	1 ♂	2 ♀	...	...	...	...	...	...	...	...
15B -	Surface soil in sheep -	12 about -	§ 55°	59°	50°	...	...	...	...	...	...	...	...	...	...	...	...
	yard	1 day old	4 1♂ 3♀	5 3♂ 2♀	3 ♀	...	...	...	...	...	...	...	...	...	...	...	...
15C -	Surface soil in sheep -	10 about -	‖ 50°	42°	35°	...	46°	44°	44°	48°	...	...	...	...	...	...	...
	yard	7 days old	...	4 2♂ 2♀	1 ♂	...	1 ♂	2 ♂ ♀	...	2 ♀	...	...	...	...	...	...	...
15D -	Surface soil in sheep -	80 adults -	§ 60°	53°	...	52°	52°	..	...	...	...	...	...	...	...	...	...
	yard		4 2♂ 2♀	12 6♂ 6♀	...	55 18♂ 37♀	7 3♂ 4♀	...	...	...	...	...	...	...	...	...	...
15E -	Surface soil in sheep -	50 adults -	§ 60°	52°	...	...	61°	...	...	...	...	...	...	...	...	...	...
	yard		3 1♂ 2♀	7 4♂ 3♀	...	31 14♂ 17♀	3 1♂ 2♀	...	...	...	...	...	...	...	...	...	...
15F -	Surface soil in sheep -	10 adults -	‖ 50°	42°	35°	...	46°	44°	44°	...	48°	47°	...	...	...	...	...
	yard		...	...	4 ♀	...	...	...	3 ♀	1 ♀	2 ♀	...	...	...	...	...	...
16A -	Tree trunk with bark.	10 1-12 hrs. old	* 54°	...	53°	53°	50°	50°	53°	...	...	52°	52°	50°	50°	...	...
	In cellar		...	...	...	...	4 1♂ 3♀	1 ♀	2 1♂ 1♀	...	...	1 ♀	...	1 ♀	...	1 ♀	...
16B -	Tree trunk with bark.	10 about -	* 54°	...	53°	53°	50°	50°	53°	...	...	52°	52°	50°	50°	...	..
	In cellar	7 days old	...	...	...	2 ♂	2 ♂ ♀	1 ♂	...	...	...	1 ♀	1 ♀	...	...	1 ♀	...

Nos. 15A, 15C, 15F, 16A, 16B, early June; 15B, early December; 15D, 15E, late November.

* Minimum Temperature (Fahr.) in Cellar. ‖ Minimum Temperature (Fahr.) in Sheep Yard. § Minimum Temperature (Fahr.) on Lawn.

No.	Position	No. and Age.	Number and sex of "ticks" which died during each following 24 hours, or fraction of 24 hours. 1	2	3	4	5	6	7	8	9	10	11	12	13	17	18
16C	Tree trunk with bark. In cellar	11 adults	* 54° ...	... ...	53° ...	53° $1_{♀}$	50° $2^{♂}$	50° ...	53° $3^{2♂}_{1♀}$	... ...	... ...	52° $1^{♂}$	52° $1_{♀}$	50° $2_{♀}$	50° ...	... ...	49° $1_{♀}$
17A	Tree trunk with bark. On lawn	12 under 1 day old	‖ 44° $3^{1♂}_{2♀}$	48° ...	47° $4^{1♂}_{3♀}$	... ...	... ...	46° $1_{♀}$	... $3_{♀}$	... $1_{♀}$	... ...	... ...	... ...	... ...	... ...	... ...	... ...
17B	Tree trunk with bark. On lawn	10 about 7 days old	‖ 35° ...	... ...	46° ...	44° $5^{1♂}_{4♀}$	44° 3	48° ...	47° $1^{♂}$	... ...	... ...	46° $1^{♂}$	... ...	... ...	... ...	... ...	... ...
17C	Tree trunk with bark. On lawn	10 adults	‖ 35° ...	... ...	46° $1_{♀}$	44° ...	44° $4_{♀}$	48° ...	47° $2_{♀}$	... ...	... ...	46° $2_{♀}$	... $1_{♀}$	... ...	... ...	... ...	... ...
17D	Tree trunk with bark. On lawn	46 adults	§ 47° $4^{2♂}_{2♀}$	50° $16^{3♂}_{13♀}$	60° $13^{4♂}_{9♀}$	52° $9^{3♂}_{6♀}$	... ...	... $1_{♀}$	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...	... ...
18	Sheep-pen sweepings - in open jar in sheep shelter	81 adults	§ 63° ...	60° $16^{14♂}_{2♀}$	53° $14^{12♂}_{2♀}$	... ...	52° $30^{9♂}_{21♀}$	52° $11^{3♂}_{8♀}$	47° $3^{1♂}_{2♀}$	50° ...	60° $1_{♀}$	52° $1_{♀}$	... ...	... $4^{1♂}_{3♀}$	61° $1_{♀}$	... ...	... ...

Nos. 16C, 17A, 17B, 17C, early June; 17D, 18, late November.

* Minimum Temperature (Fahr.) in Cellar. ‖ Minimum Temperature (Fahr.) in Sheep Yard. § Minimum Temperature (Fahr.) on Lawn.

Summary and Observations.

The foregoing Experiments may be summarised as follows:—

Experiment.	All the Louse-flies dead in			Season
	Class A. (Unfed; under 1 day old).	Class B. (Fed; 3-7 days old).	Class C. (Adults).	
	Days.	Days.	Days.	
6.—Lawn grass in celllar	11	12	12	Winter
7.—Lawn grass on lawn	6	7	9	,,
8.—Soil with leaves on surface on lawn	(a)6	8	10	,,
	(b)4	—	—	,,
9.—As above. In cellar	14	9	12	,,
10.—Soil without leaves on surface on lawn	5	(b)5	10	,,
	—	(c)7	—	,,
11.—As above in cellar	7	12	12	,,
12.—Wool on laboratory table	1	4	(c)2	,,
	—	—	(d)2	,,
13.—As above. In cellar	2	1	(c)2	,,
	—	—	(d)2	,,
14.—Box of sheep-pen sweepings in sheep shelter	7	(b)7	10	,,
	—	(c)9	—	,,
15.—On natural surface of sheep-yard	(a)7	8	(f)9	,,
	—	—	(d)5	Spring
	—	—	(e)5	,,
	(b)3	—	—	Summer
16.—Tree-trunk in cellar	14	14	18	Winter
17.—As above on lawn	8	10	(c)11	,,
	—	—	(d)6	Spring
18.—Jar of sheep-pen sweepings in sheep shelter	—	—	13	,,

These experiments show conclusively that the period of viability of Melophagus ovinus, the so-called "sheep-tick," when removed from the host is longer than has been generally believed. Curtice (1890, p. 41) found that moderately well fed "ticks" placed in a cotton-stoppered bottle, with some wool, and kept in a room with a temperature, varying between 60°F. and 80°F. all died in less than four days. Other "ticks" placed in wool over damp soil in a flower pot died within the same period. Some young "ticks," which he fed on the back of his hand were kept alive for nearly two weeks, or until their daily feeding was neglected.

Swingle (1912, p. 22) found that "ticks" kept in boxes covered with gauze all died in four days, whether they were kept warm or cold. Young "ticks," before taking a meal were kept a little longer, and in one case he was able to keep one alive for seven days.

Sweet and Seddon (1917, p. 13), kept "ticks" alive off the host for periods of eleven and eleven and three-quarter days on soil in a cellar.

In one experiment the present writer kept an adult "tick" in a cellar alive for eighteen days. In three experiments under similar conditions to the above, two "ticks," one under twelve hours old and unfed, the other about seven days old, lived for fourteen days. In two other experiments a young unfed "tick" and an adult "tick" lived for thirteen days. In the latter case the insect was kept under conditions closely simulating natural surroundings. In yet another experiment a female "tick" which was kept off the host under conditions such as might be found on any sheep run survived to the eleventh day.

Curtice (1890, p. 40) remarks of the "sheep-tick": "It is not at all probable that they can exist many days apart from the sheep as they are unfitted by structure for any other habitat." Herms (1915, p. 294), referring to this insect, says: "When off the sheep the insects die in from two to eight days, most of them dying in about four days."

Swingle (1913, p. 22) goes further, and remarks: "The 'sheep-tick' spends its whole life on the sheep. It is a false idea that the 'tick' may drop off the sheep, and live for a long time in the grass or brush, and be picked up again by sheep sometime later, as is the case with the true tick." Doubtless these statements might be applied in most cases to Australia also, but since it has been shown by Sweet and Seddon and by the present writer that "ticks" remain viable for a longer period off the host than appears to be the case in America and Europe, and in view of the well-known habit of these insects in crawling over the surface of the fleece in warm weather, it would appear very probable that some of them do become dislodged from the host and ultimately find their way back to the sheep. It must be observed, however, that in most cases "ticks" apart from the host do not die rapidly, but weaken gradually until they are no longer able to attach themselves to any object. There is a period, therefore, in the life of such "ticks" during which the individual, owing to physical weakness, would probably not be able to avail itself of an opportunity of continuing its existence on the host.

From an analysis of these experiments it will be seen that the most favourable condition and location for the survival of the adult parasite off the host was a piece of tree trunk resting on sheep-pen sweepings in the cellar in winter; the next most favourable condition was an open jar of sheep-pen sweepings in a sheep shelter in spring; the next was the lawn grass in the cellar, soil with leaves on the surface in cellar, and soil without leaves on the surface in cellar, in all three of which the parasites lived equally long. The most favourable condition was the tree trunk on sheep-pen sweepings on the lawn, where the "ticks" lived eleven days in winter and six days in spring; the next bare soil on the lawn, soil with leaves on the surface, and sheep-pen sweepings in a sheep-shelter, in all three of which the insects died in ten days during the winter. The next most favourable condition was the sod of lawn grass on lawn in winter, and the natural surface of the sheep-yard in winter, where the parasite lived nine days. Under the latter condition in spring, two groups of "ticks" all died in five days. Finally, the least favourable condition was the sheep's wool on the laboratory table and in the cellar, where the insects were all dead in two days.

Influence of age and condition on viability.—It will be seen from the above summary that generally the adult possesses greater vitality than either young fed "ticks," from three to seven days old, or young unfed "ticks," under one day old, and that "ticks" between three and seven days old possess greater vitality than those under one day old. Swingle, as has been stated, found that young "ticks" before taking a meal could be kept alive off the host a little longer than adults. In twelve experiments carried out by the present writer adults outlived unfed "ticks" under one day old; in one experiment the latter outlived the former and in one experiment both classes lived equally long. In ten experiments adults outlived young "ticks" about seven days old; in one experiment the latter outlived the former, and in one experiment they lived for an equally long period. In nine experiments young fed "ticks" between three and seven days old outlived young unfed "ticks" of less than one day old; in two experiments the latter outlived the former, and in one experiment both classes lived for an equally long period.

Influence of sex on viability.—These experiments show that female "ticks" are possessed of greater vitality than males. Both sexes were used simultaneously in forty-two experiments, and in twenty-seven of them the females survived longer than the males. In four experiments the males outlived the females and in eleven experi-

ments both sexes lived for equally long periods. Sweet and Seddon (1917, p. 11), whose observations in this direction were admittedly incomplete, observe that " . . . it is worthy of record that the last surviving 'ticks' were males.

Influence of seasonal conditions on viability.—Experiments carried out in the winter (June), and again in late spring (November) show that the adult "tick" survives outdoor longer in winter than in summer. In the first of these experiments (No. 15) the "ticks" were placed on the natural surface of the soil in a sheep-yard and covered only by a wire-gauze cage. The cage was exposed to all prevailing weather conditions excepting the direct force of the wind from one direction, and from the sun's rays during the afternoon. During the winter all of the "ticks" (ten females) died in nine days; in the early spring, when the average temperature was about 10°F. higher, thirty "ticks" of both sexes all died within five days. In the second experiment (No. 17) in which the "ticks" were kept on the lawn in a dish of sheep-pen sweepings upon which rested a piece of tree trunk, all of the "ticks" (ten females) died in eleven days in winter; in the spring, when the average temperature was about 8°F. higher, all of the "ticks" (forty-six males and females) died in six days.

6. To determine the period of viability of the pupa when removed from the host.

Experiment No. 19.

Eight pupae extruded during the morning of May 28th were kept on moist filter paper and incubated at 34°C. In all six young flies were produced on eighteenth and nineteenth days. The remaining pupae (two) were found to be dead.

Thus 75% of the pupae remained viable, and produced young flies in eighteen to nineteen days from date of extrusion.

Experiment No. 20.

Twenty pupae of unknown ages, collected on November 27th, were placed in a *dry Petri dish* and incubated at 33°C. One young fly, emerged on seventh day, one on eighth day, three on tenth day, and one on eleventh day. The remaining fourteen were found to be dead when examined on December 31st.

Thus 42.8% of the pupae remained viable, and produced young flies seven to eleven days after their removal from the host.

Experiment No. 21.

Ten pupae of unknown ages, collected on August 17th, were kept on *moist sheep-pen sweepings* and incubated at 18°C. to 28°C. Two young flies emerged on tenth day, one on seventeenth day, three on nineteenth day, two on twenty-second day, one on twenty-third day, and one on twenty-sixth day.

Thus 100% of these pupae produced young flies ten to twenty-six days after their removal from the host.

Experiment No. 22.

Thirty-five pupae, known to be less than six days old, were collected on September 19th, and kept on *moist* sheep-pen sweepings in an incubator at temperatures ranging from 26°C. to 32°C. Two young flies emerged on the twelfth day, two on fourteenth day, two on fifteenth day; six on sixteenth day; five on nineteenth day; two on the twentieth day; and one on the twenty-first day.

Thus 57% of the pupae remained viable, and produced young flies within twelve to twenty-one days of their removal from the host.

Experiment No. 23.

Thirty pupae of unknown ages were collected on August 30th, and placed on *dry* sheep-pen sweepings in an incubator at a temperature of 22°C. Two young Louse-flies emerged on fourth day, three on eleventh day, two on eighteenth day, one on nineteenth day, one on twentieth day, and one on twenty-third day. The remaining twenty-one pupae died.

Thus 30% of the pupae remained viable and produced young flies within two to twenty-three days of their removal from the host.

Experiment No. 24.

Thirty pupae of unknown ages were collected on August 30th, placed on *dry sand*, and incubated at a temperature ranging from 18°C. to 28°C. One young emerged each day from the fourth to the sixth day, two flies emerged on seventh day, two on eleventh day, two on fourteenth day, seven between the fifteenth and eighteenth days, two on nineteenth day, and one on twenty-first day. The remaining ten died.

Thus 60.6% of the pupae remained viable, and produced young flies within four to twenty-one days of their removal from the host.

Experiment No. 25.

Twenty-three pupae of unknown ages, collected on October 2nd, were placed on *dry sand*, and incubated at a temperature of 22°C. One young fly emerged on the third day, two on the sixth day, two on the tenth day, two on the thirteenth day, one on the eighteenth day, one on twenty-third day, five on twenty-seventh day, one on twenty-eighth day, two on the thirty-first day, and two on the thirty-second day.

Thus 82.6% of the pupae remained viable, and produced young flies within three to thirty-two days of their removal from the host.

Experiment No. 26.

Fifty pupae of unknown ages, collected on September 7th, were placed on *dry sand*, and incubated at 36°C. Two young flies emerged on the first day. The remaining forty-eight were found to be dead on October 18th.

Thus only 4% of the pupae remained viable, for a period of only one day.

Experiment No. 27.

Thirty pupae of unknown ages, collected on August 30th, were incubated at 22°C. on moist sand. One young fly emerged on the twelfth day, one on the eighteenth day, one on the twenty-third day, and one on the twenty-fifth day. The remaining twenty-six pupae died.

Thus 13.3% of the pupae remained viable, and produced young flies within twelve to twenty-five days of their removal from the host.

Experiment No. 28.

Forty-six pupae of unknown ages, collected on December 7th, were incubated on moist sand at a temperature of 36.0°C. None of these produced young flies.

Experiment No 29.

Forty-nine pupae of unknown ages, collected on August 30th, were placed on dry sand in Petri dish, which was placed on the lawn within the Institute quadrangle. The dish was exposed to all the prevailing weather conditions, excepting that the surrounding buildings broke the force of the wind, and that a small table which was placed over the dish prevented the rain falling directly upon the sand. On clear days the pupae were exposed to the direct rays of

the sun, excepting during the morning and late afternoon. One fly emerged on the sixth day, one on the seventh day, and one on eleventh day. The remaining forty-six pupae were found to be dead on November 5th. A thermometer exposed to the above conditions registered a maximum of 68°F., and a minimum of 44°F., during these eleven days, and a maximum of 86°F., and a minimum of 43°F., for the thirty-five days following the commencing date of this experiment.

Thus 6% of these pupae remained viable, and produced young flies from six to eleven days from date of their removal from the host.

Experiment No. 30.

The above experiment was repeated with fifty pupae collected on October 2nd. One young fly emerged on the twenty-second day, one on the twenty-fourth day, and one on thirty-fourth day. On the thirty-fourth day it was found that four pupae were missing (probably taken by birds). The remaining forty-three pupae were then examined and found to be dead.

Thus 6% of the pupae remained viable and produced young flies within from twenty-two to thirty-four days of their removal from the host. During the period the maximum temperature was 86°F. and the minimum 34°F.

Experiment No. 31.

The preceding experiment was duplicated on the same day. None of the pupae produced young flies, and all were found to be dead thirty-eight days later.

Experiment No. 32.

An experiment similar to the three preceding experiments was commenced on November 21st, with thirty-three pupae of unknown ages. None of these pupae produced young flies during the following forty days, at the end of which period they were examined and found to be dead. The maximum temperature recorded for the first eighteen days was 84°F. and the minimum 47°F. For the remaining period (twenty-six days) the maximum was 110°F. and the minimum 50°F.

Experiment No. 33.

Fifty pupae of unknown ages, collected on November 21st, were placed in a loose ball of sheep's wool about $\frac{3}{4}$ in. in diameter in a small, thin muslin bag, which was then tied to a post on the quad-

rangle lawn. The bag, which was 15 in. from the ground, was exposed to all prevailng weather conditions, as in Experiment No. 29. The range of temperature was the same as in the preceding experiment (No. 32). Three young Louse-flies emerged on the third day, one on the twelfth day, one on the sixteenth day, five on the nineteenth day, one on the twenty-fourth day, one on the twenty-sixth day, and one on twenty-seventh day. The remaining thirty-eight pupae were found to be dead on December 31st.

Thus 31.5% of the pupae remained viable, and produced young from three to twenty-seven days from the date of their removal from the host.

Experiment No. 34.

Sixteen pupae collected on August 6th were placed on *moist earth* in a Petri dish, which was kept in a cool dry cellar beneath the Institute building. Throughout the experiment the earth was kept in a moist condition. During the first twenty-five days the temperature ranged from 50°F. to 59°F., and during the remaining period of the experiment (forty-five days) the range was from 52°F. to 60°F. None of the pupae produced young flies, and all were found to be dead when examined seventy days after the experiment was commenced.

Experiment No. 35.

An experiment similar to the above, in which twenty-six pupae of unknown ages, placed on dry earth, was commenced also on August 6th. None of the pupae produced young flies.

Experiment No. 36.

One hundred and eighty pupae, collected on January 9th, were kept on the laboratory bench in a 4 oz. tobacco tin, containing a small quantity of wool. The tin was covered with its lid except when it was necessary to open it for the purpose of counting the young flies. During the experiment the maximum temperature was 90°F. and the minimum 60°F. The pupae were examined on the days mentioned, and the young "ticks" were removed from the tin on these occasions. Two young "ticks" were removed at the end of the first day, eight on the third day, sixteen on the sixth day, seven on the eighth day, eleven on the ninth day, eight on the tenth day, twenty-seven on the twelfth day, three on the fourteenth day, seven on the seventeenth day, ten on the twentieth day, five on the twenty-second day, six on the twenty-third day, four on the twenty-seventh day, three on the thirtieth day, five on the thirty-first day,

four on the thirty-fourth day, two on the thirty-seventh day, one on the thirty-ninth day, and one on the forty-second day. The remaining fifty pupae were found to be dead on February 27th.

Thus 72% of the pupae remained viable, and produced young flies in one to forty-two days after their removal from the host.

Summary and Observations.

The results of these eighteen experiments to determine the viability of the pupa when removed from the host are summarised in the following tabulated statement:—

VIABILITY OF THE PUPA.

Conditions under which pupae were kept.	Experiment No.	Range of Temperature Fahr.	First and last day on which young emerged.	Percentage of pupae which produced young.
On moist sheep-pen sweepings in incubator	21	64.40-82.40	10 - 26	100.
On dry sand in incubator	25	71.6	3 - 32	82.6
On moist paper in incubator	19	93.2	18 - 19	75.
On wool in tin in laboratory	36	60-90	1 - 42	72.
On dry sand in incubator	24	64.4-82.4	4 - 21	60.6
On moist sheep-pen sweepings in incubator	22	78.8-89.6	12 - 21	57.
In dry petri dish in incubator	20	91.4	7 - 11	42.8
In wool on post out of doors	33	47.0-84.0	3 - 27	31.5
On dry sheep-pen sweepings in incubator	23	71.6	4 - 23	31.
On moist sand in incubator	27	71.6	12 - 25	13.3
On sand on lawn	29	44.0-68.0	6 - 11	6.
On sand on lawn	30	34.0-86.0	22 - 34	4.
On dry sand in incubator	26	96.8	1 - —	4.
On sand on lawn	31	34.0-86.0	— - —	0.
On sand on lawn	32	47.0-84.0	— - —	0.
On moist sand in incubator	28	96.8	— - —	0.
On moist earth in cellar	34	50.0-59.0	— - —	0.
On dry earth in cellar	35	50.0-59.0	— - —	0.

NOTE—In all experiments in which the pupae were kept in the incubator a certain amount of humidity was provided by placing a small petri dish of water on the lower shelf.

It will be seen from this statement that under the most favourable conditions 100% of the pupae removed from the host are capable of developing into young Louse-flies. This high percentage of emergences was obtained in one experiment in which the pupae were placed on *moist* sheep-pen sweepings and kept in an incubator at

temperatures ranging from 64.4°F. to 82.4°F. The first young Louse-fly emerged on the tenth day, and others followed until the twenty-sixth day, when the last emerged.

The next highest percentage of emergences was obtained in an experiment in which the pupae were placed on *dry sand* in an incubator at a uniform temperature of 71.6°F.; under these conditions 82.6% of the pupae developed into Louse-flies, the first on the third day and the last on the thirty-second day.

The next most favourable condition for the development of the pupae was a temperature of 93.2°F., and the substitution of moist paper for dry sand. Under these conditions 75% of pupae less than one day old when gathered from the host emerged on the eighteenth and nineteenth days. A slightly lower percentage of emergence, namely, 72%, was obtained when the pupae were kept in a covered tin on the laboratory table, where the temperature ranged from 60°F. to 90°F. In this experiment the first Louse-fly emerged on the first day and the last on the forty-second day. When the pupae were kept on dry sand in the incubator at a temperature ranging from 64.4°F. to 82.4°F., 60.6% of them developed into the imaginal state—the first on the fourth day and the last on the twenty-first day. On moist sheep-pen sweepings in an incubator, at temperatures ranging from 78.8°F. to 86.9°F., 57% of the pupae experimented with produced Louse-flies, the first of which emerged on the twelfth day and the last on the twenty-first day; 42.8% of the pupae which were incubated in a dry dish at 91.4°F. produced Louse-flies, the first of which emerged on the seventh and the last on the eleventh day. The next most favourable condition for development was in wool on a post out-of-doors, at a season of the year during which the temperature ranged from 47°F. to 84°F.; 31.5% of the pupae produced young Louse-flies, the first of which emerged on the third day and the last on the twenty-seventh day. This result, it will be noticed, is of particular interest, inasmuch that the conditions under which the experiment was carried out closely simulated natural conditions. Only a very slightly lower percentage of emergences, namely 31%, was obtained in an experiment in which the pupae were placed on dry sheep-pen sweepings in an incubator at a temperature of 71.6°F. Under these conditions the first pupa developed into a young "tick" on the fourth day and the last on the twenty-third day.

In two experiments under almost natural conditions—namely, on sand on the lawn—only 6% of the pupae developed. In one of the

experiments, during which the temperature ranged from 44°F. to 68°F., the first pupa developed into a young "tick" on the sixth day and the last on the eleventh day. In the other experiment, during which the temperature varied from 34°F. to 86°F., the first "tick" did not appear until the twenty-second day and the last not until the thirty-fourth day.

The experiment which gave the lowest percentage of emergences was one in which the pupae were placed on dry sand and incubated at a temperature of 96.8°F. Under these conditions 4 % of the pupae developed into Louse-flies, namely two of a total of fifty. Both emergences took place during the first day.

Under the following five sets of conditions the development of the pupa was absolutely inhibited, namely, (1) pupae on moist sand in incubator at 96.8°F., (2) on sand on lawn at temperatures varying from 34°F. to 86°F., (3) on sand on lawn at temperatures varying from 47°F. to 84°F., (4) on moist sand in cellar at temperatures varying from 50°F. to 59°F., and (5) on dry earth in cellar at temperatures varying from 50°F. to 59°F.

General Summary and Discussion.

As is well known, the members of the family Hippoboscidae, to which the Sheep Louse-fly belongs, are all parasitic in the adult stage upon birds or mammals, and as in the other families of the Sub-order Pupipara, the larva is retained in the body of the female until it is nearly ready to transform into the pupal stage.

Unlike the true tick, which leaves the host to oviposit, the Sheep Louse-fly, or "sheep-tick," spends its whole life upon the host. The nearly fully developed larva is extruded into the wool, where it transforms, about twelve hours later, into a pupa, from which it is, however, externally indistinguishable except in hardness and colour. This pupa, or more correctly puparium, since the pupa is enclosed within the larval skin, is securely attached to the fleece by a glutinous substance which is extruded by the female with the nearly fully developed larva.

The incubation period of the pupa varies according to temperature. On sheep kept here in a stall in winter, when the temperature varied from 43°F. to 47°F., the period was found to be from twenty-two to twenty-four days. In summer, when the temperature varied from 47°F. to 72°F., the period on stalled sheep was found to be nineteen to twenty-one days.

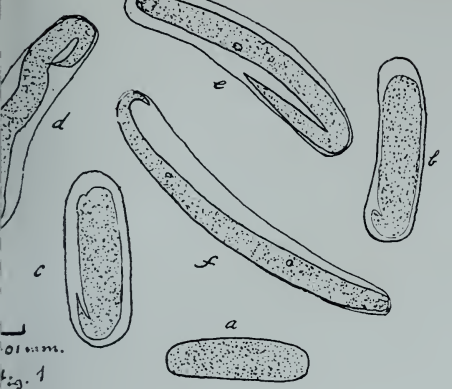


Fig. 1

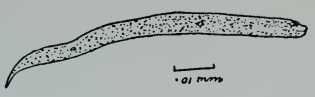


Fig. 2.

Fig. 3

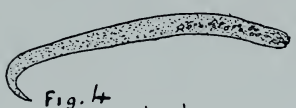


Fig. 4

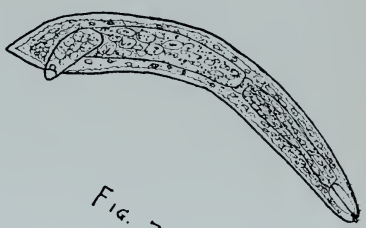
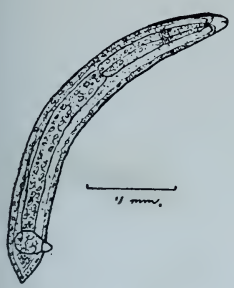


Fig. 7

Fig. 5.

Fig. 6



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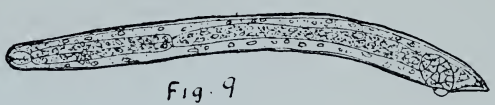

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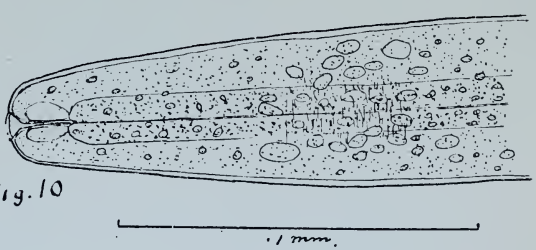


Fig. 10

Fig. 11

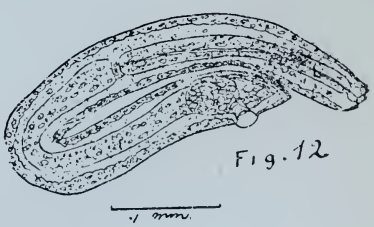


Fig. 12

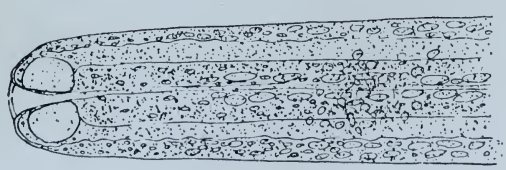


Fig. 13

Habronema muscae.

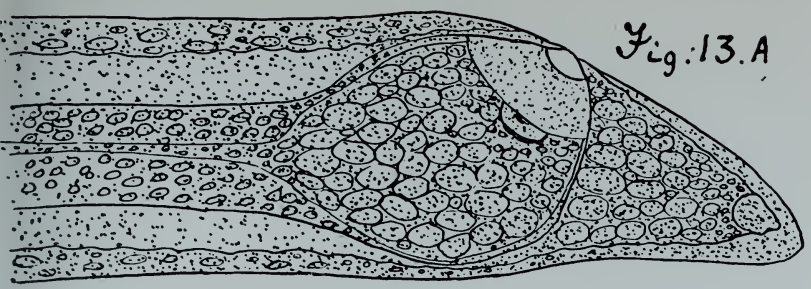


Fig. 13. A

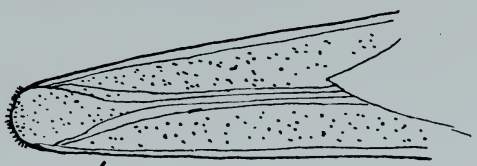


Fig. 16

.1 mm

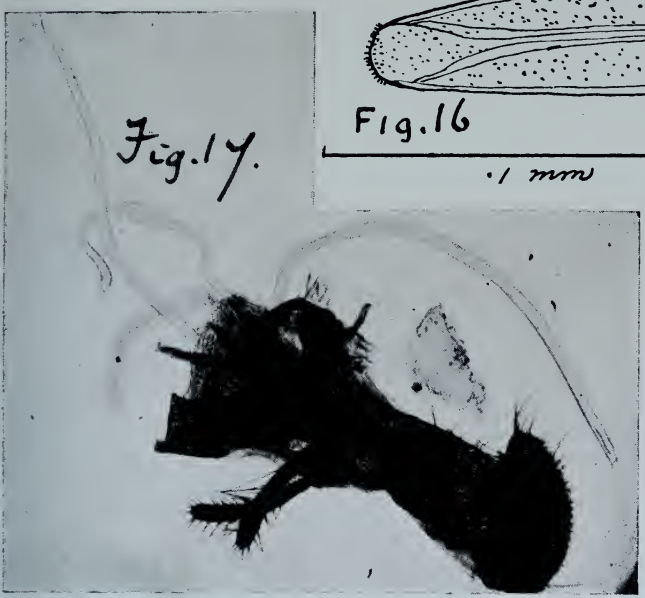


Fig. 17.

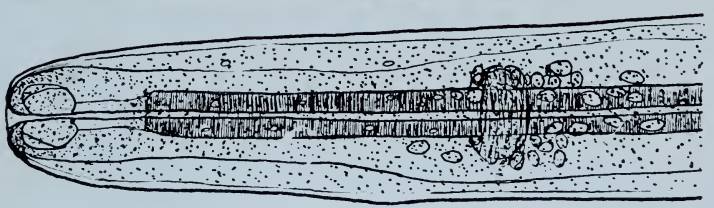
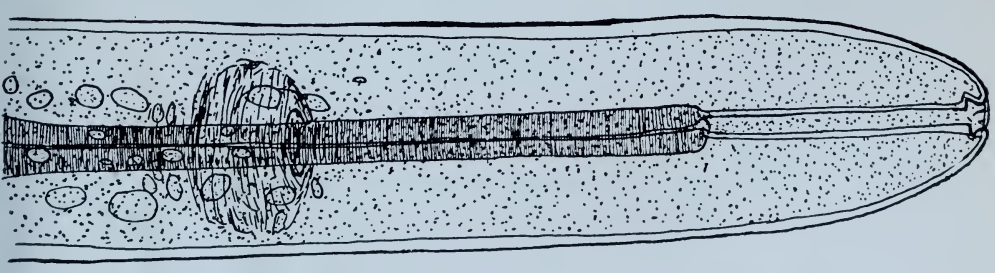


Fig 14



.1 mm

Fig. 15.

Habronema muscae.